1983

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SEASONAL VARIATION IN ALLELOPATHIC EFFECTS OF CORN RESIDUE ON CORN AND CRESS SEEDLINGS

Iowa State University PH.D. 1983

Seasonal variation in allelopathic effects of corn residue on corn and cress seedlings

by

Arnulfo G. Garcia

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Agronomy
Major: Crop Production and Physiology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

For the Major Department

Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa

1983

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INTRODUCTION

It has been established through research trials, as well as farmers' experiences, that corn yields are decreased when grown continuously. More recently, Higgs et al. (1976), Slife (1976), and Hicks and Peterson (1981) reported reduced corn yields in continuous corn compared to rotation yields. This decrease in yield persists even when fertilizers, water, pest control, and soil physical status are optimum. The decrease in yield has been associated very frequently with phytotoxins released from crop residues just separated from living plants (Nielsen et al., 1960; Lawrence and Kilcher, 1962; Guenzi et al., 1964, 1967) or from residues in the process of decomposition (Kimber, 1967, 1973a,b; McCalla et al., 1963; McCalla and Norstadt, 1974; Patrick and Toussoun, 1965; Norstadt and McCalla, 1968a,b). Results from these studies indicate that there are some substances released from one plant that could have inhibitory influence on other plants. This is known to be allelopathy. Some commonly reported allelopathic effects are reduced germination, lack of seedling vigor, seedling death, leaf yellowing, reduced tillering, and stunted or deformed roots or tops (McCalla et al., 1963; McCalla and Haskins, 1964; Patrick and Toussoun, 1965; Kimber, 1967; Rice, 1974).

Actions of allelopathy comprise mutual influence between plants on the basis of chemical substances efficient in small

amounts. These substances have to be formed in the plant and have to be excreted or liberated from them. Allelopathic influences could either be inhibitory or stimulatory in various aspects. Allelopathic influence is possible through two mechanisms, namely: (1) toxin leached directly from fresh or unweathered crop residues, and (2) toxin produced by microorganisms during residue decomposition (Guenzi and McCalla, 1962; McCalla and Haskins, 1964; Patrick and Toussoun, 1965; Toussoun et al., 1968). Rice (1974) also indicated that these substances can be liberated from plants in four primary pathways: (1) abscission of leaves and other plant parts, (2) volatilization of substances, (3) root exudation, and (4) leaching from above-ground parts.

Corn residues remaining after harvest contain water-soluble materials toxic to corn seedlings (Norstadt et al., 1967). The phytotoxic compounds are produced most abundantly in the early stages of decomposition and under anaerobic condition (Patrick, 1971). There are evidences that the decomposition of plant residues in the soil results in the formation of compounds which may have either favorable or unfavorable effects on plants. These compounds released during the decomposition may be the key reason why succeeding yields of corn are reduced when corn is grown continuously on the same area.

The objectives of this study were (1) to investigate

the seasonal variation in allelopathic effect of corn residues left after harvest on the growth of corn seedlings under varying tillage practices, and (2) to investigate how these allelopathic substances are released from the corn plant or corn residues.

LITERATURE REVIEW

Allelopathic Effects of Plants

Water extracts from tops, roots, or seeds of a number of crops and weed species have been found to inhibit germination or early seedling development. More recently, extracts of both vegetative parts and seeds of certain crop species have been shown to be phytotoxic to seedling development. Extracts of the vegetative portion and plant residue of crown vetch (Coronilla varia L.) incorporated in the soil were found to be toxic to crownvetch seedlings (Beiber and Hoveland, 1968). McKee et al. (1971) also found that water-soluble leachate of crownvetch seeds caused abnormal seedling development in most of the 48 species tested. Cope (1982) reported that leachate from seeds of eight forage species inhibited seedling growth and germination of crownvetch. However, he said that inhibition of germination was not a good measure of phytotoxicity since seedling growth was often inhibited when germination was not.

Overland (1966) showed that water-soluble root exudates of barley caused inhibition of germination and growth, thereby indicating an inhibitory substance or substances present. She also observed that there was a definite concentration effect and suggested periodic production of the inhibitor. Living plants and exudates of living roots were more inhibi-

tory than aqueous leachate of dead roots, which supports the concept of an active metabolic secretion of allelopathic substances.

Nielsen et al. (1960) demonstrated that corn plants contain water-soluble compounds that inhibit seed germination and seedling growth of several crop plants including corn. Aqueous extracts of mature corn stovers decreased shoot and root growth of alfalfa, timothy, corn, oats, and potato.

Tamura et al. (1967, 1969) stated that it has been well known that red clover (Trifolium pratense L.) exhibits allelopathy against itself. Chang et al. (1969) concluded that "clover sickness" results from the exudation by red clover of isoflavonoids which decompose to phenolic compounds that accumulate in the soil to an inhibitory level.

Chandramohan et al. (1973) isolated vanillic acid, p-hydroxybenzoic acid, p-coumaric acid, and three unidentified phenolic acid from rice field soil in South India. They reported that cinnamic acid (a related compound) was inhibitory to growth of rice seedling even at 0.0001 M concentration. From their study, they observed also that nitrogen application to the soil decreased the toxicity caused by the phenols.

Patterson (1981) reported that, at concentration of 10^{-3} M, caffeic, transcinnamic, p-coumaric, ferulic, gallic, and vanillic acids significantly reduced dry matter production, leaf expansion, plant height, leaf production, net assimila-

tion rate and leaf area duration on a 3-week-old soybean grown in aerated nutrient solution. However, at 10^{-4} M concentration, none of these compounds inhibited growth. This indicates that these compounds are only inhibitory at certain concentrations.

Hussain and Gadoon (1981) observed that Sorghum vulgare Pers, a tropical fodder crop, significantly reduced the vertical growth and dry mass of S. vulgare, Pennisetum americanum, Zea mays, and Setaria italica. Aqueous extracts of various plant parts, field soil, and decaying mulch significantly reduced germination, radicle growth and water contents of all test species. Toxicity levels were observed to be dependent upon the plant parts used in the bioassay experiment.

Aqueous extracts from leaves of giant ragweed (Ambrosia tifida) reduced growth in sorghum seedling and reduced germination in sorghum and radish. Five phenolic compounds were isolated and characterized but not identified. The germination bioassay using these compounds demonstrated a decrease in germination and that the decrease in germination is related to concentration (Rasmussen and Einhellig, 1980). Groves and Anderson (1981) reported that aqueous leaf extracts of Artemisia tidentata significantly reduced shoot and radicle growth of A. cristatum and radicle growth of Elymus cinereus.

In the study conducted by Mueller-Wilmes and Zoschke (1980) on the continuous growing of winter barley, they observed that a 5-year average in field trials, one barley after another, showed a reduction in grain yield of 18% with 0 kg N/ha and 5% with 100 kg N/ha compared to the first winter barley. The grain yield decline in continuous barley was caused by a decrease in the root formation at heading time. The decrease in root formation resulted in the reduction of root dry matter and of living roots, which are active in nutrient uptake. Phenolic compounds were regarded as the possible cause of root reduction at heading stage. These compounds were observed to increase during shooting and heading stage and caused the different root formation at the earing period. This study indicates a seasonal variation in the release of allelopathic compounds during barley growth and development.

Another study that showed seasonal variation in phytotoxicity is the work done by Carballeira and Cuervo (1980). The seasonal variation in the phytotoxicity of aqueous extracts obtained from soils collected at two depths, 0-10 and 10-20 cm, from heathland dominated by the species Erica australis L., in the months of April, October, and January, was studied on the Avena coleoptile straight growth test and germination assay using seeds of the following grassland species: Trifolium pratense L., T. ripens L., Phleum pratense

L. and Lolium perenne L. The bioassay indicated a considerable phytotoxicity in the soil collected in April. Protocatechic, vanillic, p-hydoyxybenzoic and p-coumaric acids were identified as the possible phytotoxin in the extracts.

Crop Residues as Source of Allelopathic Substances

Accumulated evidence has indicated that toxic substances are produced in the decomposition of crop residues in the soil. Guenzi and McCalla (1962) collected crop residues of wheat and oat straw, soybean and sweet clover hay, corn and sorghum stalks, and bromegrass and sweet clover stem in September from the field. These crop residues were extracted with hot and cold water using 1 part to 15 parts water and the extracts were used to test their effect on germination and growth of wheat, sorghum, and corn. They observed that all crop residues contained water-soluble substances that depress plant growth of wheat, corn, and sorghum. Guenzi and McCalla (1966) identified and quantified five phenolic acids in mature plant residues of oats, wheat, sorghum, and corn. These five compounds were p-coumaric, syringic, vanillic, ferulic, and p-hydroxybenzoic acids. They estimated on the basis of usual yields of the four crop plants that the following amounts of p-coumaric acid, which is present in the greatest amount, would be added in pounds per day by the residue: 89 by sorghum, 72 by corn, 8 by heat, and 23 by

oats. They pointed out that, even though these acids are mostly bound in the residues, there should be a period during decomposition when rather large amounts could be released in the immediate vicinity of the residue and be sufficiently high to affect plant growth.

Guenzi et al. (1967) investigated changes in phytotoxic activity of water extracts of corn, wheat, oats, and sorghum residues during decomposition in the field. They observed that toxicity of extracts from wheat straw remained about the same through the first four weeks of decomposition but toxicity disappeared by 8 weeks. The greatest toxicity in extracts of oat straw residue occurred at harvest time and essentially all inhibitory activity was gone after 8 weeks of decomposition. The extracts from sorghum residues increased in toxicity up to 16 weeks and then declined in toxicity. Toxicity from extracts of corn residues remained high during 22 weeks of decomposition but decreased rapidly thereafter. The study by Assumpcao (1979) demonstrated that corn residue inhibited corn seedling growth.

Chou and Patrick (1976) identified 18 compounds in decomposing corn residues in soil: salicyaldehyde, resorcinol, phloroglucinol, p-hydroxybenzaldehyde, butyric, phenylacetic, 4-phenylbutyric, benzoic, p-hydrobenzoic, vanillic, ferulic, o-comaric, o-hydroxyphenylacetic, salicylic, syringic, p-coumaric, transcinnamic, and caffeic acids. Most of these

compounds are phytotoxic in lettuce seed bioassay. Pareek and Gaur (1973) also found that concentrations of vanillic, p-hydrobenzoic, p-coumaric, salicylic, and syringic acids were considerably higher in rhizosphere soils of Zea mays than in nonrhizosphere soils. Dzubenko et al. (1977) reported that toxin increased in quantity in the roots and in the rhizosphere when crop plants of various types were grown in continuous monoculture. Moreover, the increase in plant and soil toxicity was correlated with a decrease in crop productivity.

Crookston and Kurle (1982) investigated the effect of above-ground corn residue on the following corn yield. They observed that removing the above-ground corn residue from a previously planted corn plot did not prevent yield decrease of the following corn plant. Their study indicated that the allelopathic compounds in corn plants are not coming from the above-ground corn residues. However, the study conducted by Varley and Cruse (1982) on the early growth of corn at three temperature regimes (13°C, 18°C, and 24°C) showed that residues placed near or with the seed and residues distributed throughout the top 12.5 cm of the soil had the strongest inhibition on corn seedling growth at all temperature regimes. Yakle and Cruse (1981) observed that corn root and shoot weights were more reduced by fresh corn residue than by the partially decomposed corn residue. The effect

was more noticeable when the corn roots came in direct contact with the residue layer.

Since unharvested parts of rice plants are customarily mixed with the soil, Chou and Lin (1976) investigated the effect of decomposing rice residue in soil on the growth of rice plants. They observed that aqueous extracts of decomposing rice residues in soil inhibited radicle growth of rice, Oryzae sativa L., and lettuce seedlings, as well as growth of rice. Maximum toxicity occurred during the first month of decomposition and then declined thereafter. Five phytotoxin, p-hydrobenzoic, p-coumaric, vanillic, ferulic, and o-hydroxyphenylacetic acids, were identified from decomposing rice residues under waterlogged conditions. Kuwatsuka and Shindo (1973) previously identified some of the same phenolic acids in decaying rice residues and in rice straw.

Chou et al. (1981) reported that phytotoxicity during decomposition of rice straw in soil was highest at 20-25°C. Temperature above 25°C enhanced rice straw decomposition and degraded the phytotoxic substances more rapidly. Five phytotoxic phenolics were obtained from both aqueous extracts and residue of the incubated soil samples. It was observed that phenolics were likely higher in the samples incubated at 25°C than at 15 or 35°C. The quantity of toxin released during decomposition of rice straw in soil reached highest levels 6 weeks after incubation and gradually disappeared after 12 weeks.

Patrick et al. (1963) reported that the majority of the toxic materials were confined to the decomposing residues. They found that phytotoxicity to lettuce was most severe during the first 10 to 25 days of residue decomposition and then started to diminish with increasing period of decomposition. The most severe phytotoxicity occurred fields when decomposition of plant organic matter had taken place in cold, wet soil and during the early stages of decomposition.

Kimber (1973b) conducted an experiment to verify whether the depressed yield of subsequent wheat crops having wheat residues was due to the immobilization of nitrogen by the increased population of soil microflora. His work showed that both nitrogen immobilization and toxin affect the yield of wheat when grown in the presence of excess straw residue. Germination appeared to be depressed to the greatest extent by straw laying on the surface of the soil, whereas nitrogen immobilization affected yield the most when the straw was mixed into the soil. He pointed out that the addition of nitrogen did not overcome the effect of the straw. Tang and Waiss (1978) reported that the major compounds produced during wheat straw decomposition were salts of acetic, propionic, and butyric acids. The amounts increased gradually up to 12 days and the toxicity of the straw extracts to wheat seedlings increased accordingly.

Phytotoxicity from decomposing surface wheat residues

were observed to contribute to poor seedling growth (Cochran et al., 1977). Growth of seedlings were retarded most severely in areas of the field where chaff or straw were concentrated. Crop residue decomposing under cool, wet conditions may release short chain fatty acids which may inhibit germination and retard seedling growth of wheat (Elliot et al., 1981; Lynch, 1977, 1978). Moreover, Cochran et al. (1977) observed that winter wheat seedlings growing in heavy surface residue often set a high crown node which exposes the adventitious roots to phytotoxin produced during decomposition of the straw. They also reported that residues of lentil, pea, wheat, barley, and bluegrass produced wheat seedling root inhibitors but only after conditions became favorable for microbial growth. Toxin production from these straws were generally preceded by wet weather with temperatures above freezing but below 15^{o}C. However, a recent report by Cochran et al. (1982) involving a three-year study on winter wheat showed that there was no water-soluble phytotoxin from surface crop residue and that winter wheat yield from plots direct drilled into surface cereal residue was equal to that from plots tilled and seeded conventionally.

Effect of No-tillage Practice on Soil Ecosystem

There is concern by many farmers that no-tillage planting of corn might create a slow and uneven emergence which

will slow crop growth. Elliot and Lynch (1982) reported that phytotoxicity from decomposing surface residues has been one of the constraints on the success of no-till seeding. However, the study by Eckert (1982) showed that no-tillage corn could be planted during the early portion of the optimum planting date despite the cooler soil temperature associated with the system and the yield is comparable to that of conventional tillage. Additional moisture available to no-till corn grown on well-drained soil compensates for the retarded early growth and normally allows the crop to develop at a rate comparable to conventionally tilled corn.

Several changes in the soil ecosystem are associated with reduced tillage and residue management, as compared to conventional tillage. The soil is wetter and cooler during period of active growth of the crop. Maintenance of crop residues at the soil surface plays an important role in the soil chemical environment and in the rate at which nutrients become available to crop plants and other life forms in soil (Power and Legg, 1978). Very little is known about the effect of surface residue accumulation on soil microflora. McCalla et al. (1962) found microorganisms were more numerous within the top 2.5 cm of soil where residues are conserved by sub-tilling as compared to plowed soil. Elliot et al. (1978) demonstrated that crop yield can be reduced by microbial production of phytotoxic substances where surface residues

are maintained.

Carter and Rennie (1982) also observed that at 5-8 cm depth in no-till, microbial biomass was enriched. Doran (1981) and Doran et al. (1982) reported that microbial biomass levels and population of aerobic and anaerobic microorganisms in the surface 50 to 75 mm of no-tilled soils aver- 30 to 50% higher than those of conventional tillage. Unlike conventional-tilled soils, where microbial activity is often greatest at a depth of 75 to 150 mm, levels of microbial biomass and aerobic microbial activity rapidly decline in no-tilled soils below 75 mm depth. The microbial domains produced by tillage practices are closely related to the physical changes in the amount and distribution of water and organic substrates and the biological availability of oxygen. The higher bulk density and soil-water contents associated with no-till practice, as compared to the conventional tillage, can restrict oxygen diffusion below 75 mm depth, resulting in decreased potential for nitrification and increased denitrification.

In an 18-year study conducted by Dick (1982) on enzyme activity between no-till and conventional tillage, he found that enzyme activities were 2-5 times greater in the surface layer (0-1.25 cm) of the no-till plots compared to the conventional-tilled plots and significantly less in the 22.5-30 cm soil layer. The enzyme activities were correlated with

organic C content and with soil pH.

Role of Microorganisms in the Decomposition of Allelopathic Compounds

It is obvious that, if allelopathic compounds which are released into the environment were not decomposed, probably no plants could ever survive. Once the phytotoxins are produced and escape into the environment, they begin to be decomposed by microorganisms or by chemical action not involving microorganisms.

Henderson and Farmer (1955) and Henderson (1956) did an elaborate study on decomposition of various phenolic compounds by soil fungi. Several fungi isolated from soils under a variety of vegetational types were found to attack p-hydroxybenzaldehyde, ferulic acid, syringaldehyde, and vanillin. These compounds were used as the sole source of carbon by the microorganisms tested. Vanillin and ferulic acid were converted to vanillic acid before the breaking down of the benzene ring, syringaldehyde was converted to syringic acid, and p-hydroxybenzaldehyde was converted to p-hydroxybenzoic acid. These intermediate products were found to be attacked by adaptive enzymes, and the formation of these enzymes was greatly decreased by antibiotic citrinin. All the intermediate products are toxic, so allelopathic effects could continue to be exerted for a prolonged period, even during decomposition. In addition, considerable amount of

the original phenolics remained after 24 days under favorable growing conditions for the fungi involved.

Several species of Pseudomonas have been reported to decompose gallic acid, which is often found free in many plants and which results from decomposition of gallotannins (Tack et al., 1972). Kunc (1971) investigated decomposition of vanillin in chernozem soil and reported it was decomposed via vanillic acid and protocatechuic acid before the aromatic ring opens. It was observed that the number of bacteria capable of using vanillin as the sole carbon source increased. Of the 21 strains isolated, 15 were identified as Pseudomonas sp., 5 as Cellulomonas sp., and one as Achromobacter sp.

Turner and Rice (1975) studied the amount of ferulic acid, which is one of the compounds resulting from decomposition of lignin and thus an important phenolic compound, that accumulates in soil under hackberry. They observed that over 99% of the extractable ferulic acid was lost from decaying hackberry leaves in 300 days. Eighteen microorganisms, mostly belonging to the genus Pseudomonas, were found to be able to use ferulic acid as the sole source of carbon. It also was observed that many species of microorganisms were actively growing where the amount of ferulic acid in the soils are high. Black and Dix (1976) also reported the ability of 21 species of fungi to use ferulic acid as a sole source of carbon.

MATERIALS AND METHODS

Growth Chamber Experiments

Experiment 1

During the second week of November 1981, soil samples from a newly harvested corn field and fall plowed were collected at the Agronomy and Agricultural Engineering Research Center, west of Ames, Iowa. The soil samples (Nicollet clay loam), which were collected from 0 to 15 cm depth, included corn residues left after harvesting. The soil samples were then stored in a cold room with a $4^{o}C$ temperature. Soil samples that were used as control treatments were soils collected from a fallow field with very small amount of residue. These soil samples were then used to bioassay corn growth inside the growth chamber.

For this experiment, the following treatments were used:

1. Fallow soil which served as the control
2. Fallow soil plus coarse above-ground corn residues
3. Fallow soil plus fine above-ground corn residues
4. Soil with corn residue that were collected from a newly harvested corn field.

For treatments 2 and 3, above-ground corn residues which were collected from a bale of corn stover in the field were ground into coarse and fine residues. These fine and coarse residues were then mixed with fallow soil at the mixture rate of 5 g of the corn residue per kg of fallow soil. This was

equivalent to about 10,000 kg of residue per hectare of soil.

Each of the four treatments were then mixed with sand at the rate of 2:1 mixture of soil and washed sand using a cement mixer. Mixed grade NPK fertilizer (6-10-4) was also incorporated into each soil treatment at the rate of 200 kg N, 330 kg P, and 130 kg K per hectare.

Using small white plastic pots (10x15 cm), 1 kg of soil: sand mixture for each treatment was placed in each plastic pot. Each treatment was replicated 10 times using one plastic pot per replicate. One seed of A619xA632 single cross hybrid corn cultivar was then planted in each pot at a depth of 5 cm. The pots were then placed inside the growth chamber. The lighting inside the growth chamber was adjusted to give 16 hours per day with approximately 140 μEinstein $m^{-2}sec^{-1}$ and 8 hours of dark. The day temperature was $26^{o}C$ and night temperature was $20^{o}C$. Relative humidity ranged from 60 to 95% throughout the experiment. Watering of pots to near field capacity was done daily.

For this experiment, planting was done on January 25, 1982 and corn plants were harvested on February 24, 1982.

The following parameters were measured in this experiment:

1. Weekly height of each plant
2. Fresh and dry shoot weights of each plant after four weeks of growth
3. Fresh and dry root weights of each plant after four weeks of growth

4. Fresh and dry biomass weights of each plant after four weeks of growth
5. Fresh and dry root:shoot ratio (by weight) after four weeks of growth.

Corn seedling plant heights were measured from the soil level to the tip of the longest leaf at one, two, three, and four weeks after planting. Four weeks after planting, the plants were harvested and fresh shoot and root weights were recorded. At harvest, the fourth leaf had fully emerged. The biomass weight was obtained by adding the root and shoot weights. After getting the fresh shoot and root weights of each plant, the samples were then dried in a forced air drier at 50°C for three days, after which dry shoot and root weights were recorded. The root:shoot ratio was then computed by dividing the root weight by the shoot weight for both fresh and dry weights.

Statistical analysis using a randomized complete block design was used in this experiment. Significant differences between treatment means were tested using Duncan's multiple range test at 5% level of probability.

Experiment 2

This experiment was conducted in order to verify whether nitrogen level in the corn-residue soil is the limiting factor. The result of this experiment also showed the level of nitrogen to use in the succeeding experiments.

A split-plot design was used in this experiment. Two soil groups were used as the main plot and three levels of nitrogen as the split-plot. The two soil groups, fallow soil (control) and soil with corn residue, were both the same soils as used in Experiment 1. The three levels of nitrogen were 100, 200, and 300 kg N/ha.

The following were the treatments used in this experiment:

1. Soil with corn residue plus 100 kg N/ha
2. Soil with corn residue plus 200 kg N/ha
3. Soil with corn residue plus 300 kg N/ha
4. Soil without corn residue plus 100 kg N/ha
5. Soil without corn residue plus 200 kg N/ha
6. Soil without corn residue plus 300 kg N/ha.

Mix grade of NPK fertilizer (6-10-4) was used as the source of nitrogen fertilizer. As in Experiment 1, each treatment was mixed with sand at a combination of 2:1 soil and sand. The different nitrogen rates were then incorporated into the different treatments. Using the same size of plastic pots as in Experiment 1, one kilogram of soil of each of the different treatments was placed in each plastic pot. Each treatment was replicated six times with one plastic pot as one replicate. Using the same corn hybrid as in Experiment 1, one seed was planted 5 cm deep in each plastic pot. The pots were then placed inside the growth chamber with the same environment as described earlier.

For this experiment, planting was done on March 2, 1982 and corn plants were harvested on April 2, 1982.

The same parameters as in Experiment 1 were measured in this experiment. Statistical analysis using a split-plot design was done. Significant differences between treatment means were determined using a Duncan's multiple range test at 5% level of probability.

Experiments 3 to 8

In this series of experiments, monthly soil samples (Nicollet clay loam) with three different tillage practices were collected from a field of corn which had been planted the previous season at Lippert Farms, Ames, Iowa. Soil samples were collected every 25th to 28th day of the month starting from April to September 1982. The three different tillage practices were no-till, disc, and plow. For the no-till, disc, and plow treatments, soil samples were collected at depths of 5, 10, and 20 cm from the soil surface, respectively. The purpose for collecting soil samples at various depths was that the corn residues were incorporated in the soil to these proportional depths by the tillage treatments. These soil samples were then bioassayed by growing corn inside the growth chamber.

The Iowa State University soil tests of the April soil sample for the no-till, disc, and plow treatments were 119, 43, 43 kg P/ha, 544, 408, 220 kg K/ha, 7.75, 7.70, 7.70 pH,

and 5.0, 5.0, 4.3% organic matter, respectively. Nitrate plus ammonium was determined in the KCl extracted soil by a micro-Kjeldahl procedure. The no-till, disc, and plow treatments have 76, 222, 113 kg/ha of nitrate and ammonium.

Soil samples collected during the months of April, May, June, July, August, and September corresponded to Experiments 3, 4, 5, 6, 7, and 8, respectively.

The treatments used in these experiments were:

1. Control (fallow soil)
2. No-till
3. Disc (spring disc)
4. Plow (fall plow).

Soil samples for each of the different tillage practices were collected from six replicate plots in the field. These six replicated soil samples were then mixed together representing each treatment. Since the soil samples collected from the field were compact and usually in big clods, especially towards the end of the season, they were ground using a big rolling pin. The soils for the control treatment were the same soils used in Experiment 1.

Using the same methodology as in Experiment 1, the different treatments were mixed with sand at a 2:1 soil and sand mixture. Two hundred kilograms N per hectare was applied using a mix grade NPK fertilizer (6-10-4). The same plastic pot size with one kilogram of soil for each treatment and

replicate was used. Each treatment was replicated 10 times. Corn seeds (same hybrid as in Experiment 1) were planted at the rate of one seed per pot. The pots were then placed inside the growth chamber with the same care and environment as described in Experiment 1.

Two days after every monthly soil collection in the field, planting of corn in the growth chamber was performed. Harvesting of the corn plants was done four weeks after planting. At this stage, the fourth leaf of the corn plants had all fully emerged.

The parameters that were measured for each treatment were the same as in Experiment 1. A randomized complete block design was used in the statistical analysis of the data gathered. Duncan's multiple range test was used to determine significant differences between treatment means at 5% level of probability.

In order to determine the monthly variation of the different tillage practices, the different parameters measured for each month were then expressed as a percentage of the control. Statistical analysis using a split-plot design was done with month as the main plot and the different tillage practices as the split-plot.

Experiment 9

An existing corn field planted with Pioneer 3780 at Berkey Farm, west of Ames, Iowa, was the source of soil samples (Nicollet clay loam) for bioassaying corn growth in

this experiment. On June 30, 1982, a 6 m x 3 m corn plot was covered with black plastic. The reason for covering the soil with black plastic was in order to prevent the rain-leached substances and senescing above-ground plant parts to get into the soil. In this case, the only possible source of allelopathic substances would then be coming from the roots. The plots were replicated four times in the field. Four similar plot sizes, except that they were not covered with black plastic, were also measured and were the source of soil samples for the uncovered treatments. Rain-leached substances, senescing above-ground plant parts, and root exudates were then the only possible sources of allelopathic substances for the uncovered treatments.

The following were the treatments used in the experiment:

1. Control (fallow soil)
2. Covered soil with samples collected between two corn rows
3. Covered soil with samples collected under the corn plant
4. Uncovered soil with samples collected between two corn rows
5. Uncovered soil with samples collected under the corn plant.

For treatments 2 and 4 (covered between and uncovered between), soil samples were collected between two rows of

corn plants. The distance between rows was 70 cm. For treatments 3 and 5 (covered under and uncovered under), soil samples were collected within the rows of corn. Caution was maintained during the process of soil collection so as not to include corn roots in the samples. For all treatments, except the control, soil samples were collected from the upper 10 cm of the soil surface. Soil samples were collected on October 28, 1982, just a week before the corn plants in the field were harvested. For the control treatment, the same soil sample as in Experiment 1 was used.

Since the soil samples collected from the field were hard and in big clods, they were ground into finer particles using a big rolling pin. The four replicated soil samples for each treatment were then mixed together.

Using the same methodology as in Experiment 1, such as fertilizer rate, soil:sand mixture, and planting of one seed per pot, the pots were placed inside the growth chamber having the same environmental conditions as described before. Each treatment was replicated 10 times. Planting of corn was done on November 2, 1982, and harvesting of the corn plants was done on December 3, 1982.

The same parameters as in Experiment 1 were measured in this experiment. A randomized complete block design was used in the statistical analysis. Significant differences between treatment means were determined using Duncan's multiple range

test at 5% level of probability.

Resin Column Experiments

Experiments 10 to 15

Using the same soil samples collected monthly in Experiments 3 to 8, another series of experiments was done in the laboratory. The method on how soil samples for each treatment were collected was described earlier in Experiments 3 to 8. Soil samples collected from April, May, June, July, August, and September corresponded to Experiments 10, 11, 12, 13, 14, and 15, respectively.

The main-plot treatments were months of sampling and the split-plot treatments were the following:

1. Control (fallow soil)
2. No-till
3. Disc
4. Plow

In each experiment, 300 grams of each soil sample were mixed with sand at a 1:1 (w/w) soil:sand mixture. Mix grade NPK fertilizer (6-10-4) was also incorporated into the soil: sand mixture at a rate of 200 kg N/ha. Each soil sample was then placed in a container made from a 1-liter polyethylene plastic bottle with the bottom removed.

Resin column preparation XAD-4 resin, purchased from Mallinckrodt, was used in this experiment. Amberlite XAD-4

is a hydrophobic styrene-divinyl benzene co-polymer with a specific surface area of 750 m^2/g. Hydrophobic or partially hydrophobic substances are selectively retained by the XAD-4 resin. Since XAD-4 resin was contaminated with aromatic impurities, it was cleaned with hot running water for 8 hours followed by soaking in acetone for 24 hours. The cleaned resin was then stored in methanol and placed inside the refrigerator.

The resin column was prepared using a 18 x 150 mm columm which was packed with approximately 12 g of XAD-4 as an aqueous slurry. The residual methanol was removed by washing the column with 10-bed volumes of deionized water.

Collection of allelopathic substances As shown in Figure 1, the resin column and the circulating attachment were then connected to the bottom of the containers through a bored rubber stopper. Polyethylene tubings were used as the circulating attachment. The soil:sand mixture were then saturated with deionized water. The solution was circulated at a rate of 1 liter per hour by airlift. Water was replenished about twice a day to compensate for aspiration and evaporation loss. The setup which was replicated twice for each soil sample was placed inside a dark room with a temperature of 21-22^{o}C. This method of collecting allelopathic substances from the soil samples through XAD-4 resin column was patterned after the methodology used by Tang and Young (1982).

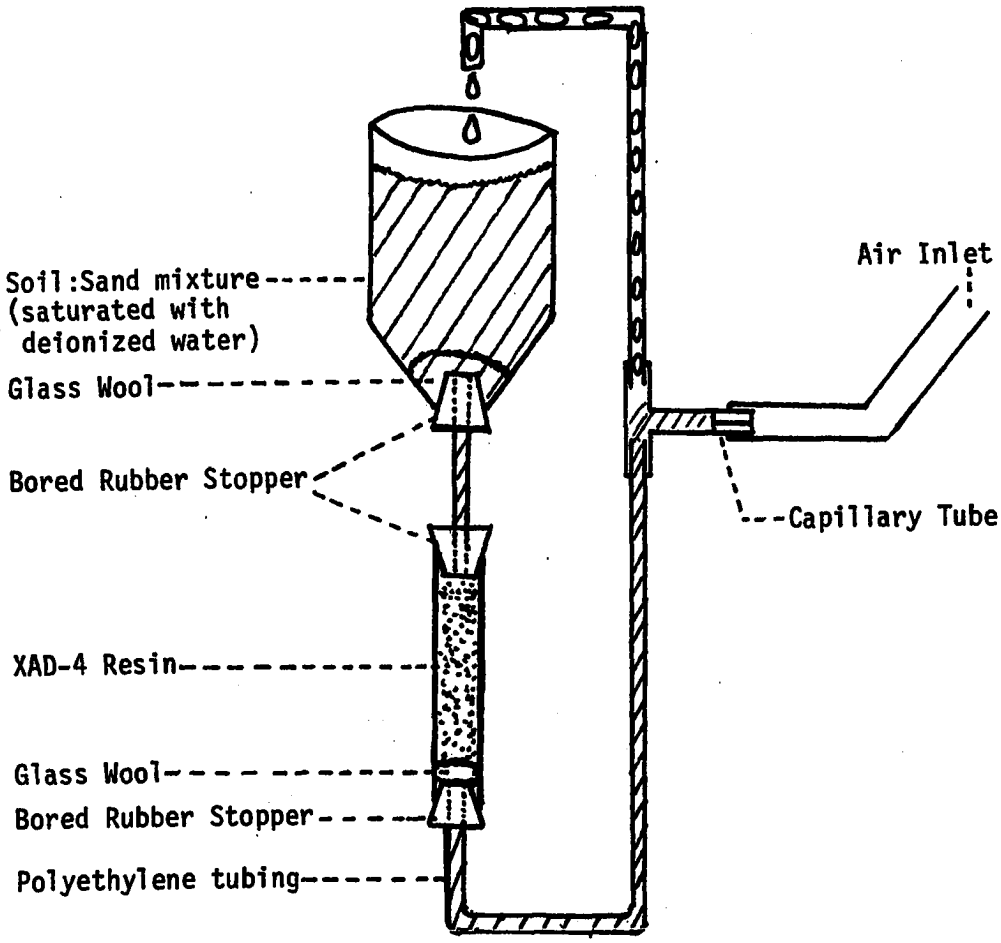

Figure 1. Diagram of the XAD-4 resin column attached to the circulating mechanism for each of the treatments with different soil samples; XAD-4 resin serves as a trap for the allelopathic substances (patterned after Tang and Young, 1982)

For each soil sample, the resin column was replaced three times. The first resin column was attached to the circulating mechanism for only one day. Then it was replaced by a new resin column and left attached for three days. After three days of the second resin column, it was replaced with the third column for an additional six days.

Extract preparation The resin columns which were detached after 1, 3, and 6 days were then eluted with 60 ml methanol. The process of getting the eluates was that each resin column was eluted with 15 ml of methanol and then centrifuged at 1000 x gravity. This procedure was repeated three more times.

The methanol was then evaporated to dryness using a rotary flash evaporator. The residue in the flash was dissolved with 5 ml of deionized water. These extracts were then bioassayed by their effect on cress seed germination.

Cress seed germination bioassay Cress seeds (Lepidium sativum cv. Curly cress) were used in the germination bioassay in this series of experiments. Plant growth analysis was used to quantify the simple bioassay response of cumulative seed germination. In some studies, this function approach to plant growth analysis had been applied to cumulative germination data. Novel indexing of the Richards' function fitted to cumulative seed germination curves was used as a bioassay technique to demonstrate the inhibitory effect of the differ-

ent extracts containing allelopathic substances. This methodology was derived from the study conducted by Lehle and Putnam (1982).

The treatments consisted of 100 cress seeds per petri dish imbibed and incubated in the dark at 25°C for 24 hours. The cress seeds were placed in a covered 9-cm petric dish with a single sheet of Whatman No. 1 filter paper moistened with 4 ml of test solution or deionized water (control). Four extract concentrations were used, namely: 0, 0.5, 1.0, and 2.0 ml of extracts. The final volume of the test solution was raised to 4 ml by the addition of deionized water. After 24 hours in the dark, the petri dishes were exposed to continuous fluorescent light. Percentage germination was recorded twice daily at an interval of 12 hours. Seeds were recorded as germinated if the radicle exceeded the length of the longest seed coat dimension. Seeds were removed from the dishes upon reaching germination.

Curve fitting and germination index calculation The Richards' function was fitted to cumulative cress seed germination curves for each treatment and replicate separately using the nonlinear regression program of Statistical Analysis System (SAS), which utilizes the Marquardt's method of sum of squares minimization. The nonlinear regression program is shown in the Appendix. The program requires initial values for all the parameters to be estimated. Shown in Appendix

Table A1 are the initial values and the allowable range for each parameter to be estimated.

Following the nomenclature used by Richards (1959), the forms of the function were defined as:

$$W = A(1 + be^{-kt})^{1/1-m} \quad \text{for } m > 1 \qquad (1)$$

$$W = A(1 - be^{-kt})^{1/1-m} \quad \text{for } m < 1 \qquad (2)$$

where W was the cumulative cress seed germination at time t, in days, after the onset of imbibition. The parameters A, b, k, and m were empirically derived constants unique to each asymptotic curve. Of these parameters, only A had any direct biological interpretation in that it represented the cumulative cress seed germination percentage as $t \to \infty$, i.e., the final cumulative germination percentage.

Using the derived parameters for each replicate, several biological meaningful variables were calculated. The weighted mean cumulative germination rate (R) was defined according to Richards as:

$$R = Ak(2m + 2)^{-1} \qquad (3)$$

Using the style of Lapp and Skoropad (1976), the onset of germination ($t_{0.01A}$) was defined in terms of the time required to reach 1% of the final cumulative germination percentage (A) by substituting W = 0.01A into either equation 1 or 2 and solving for t. The equations obtained were:

$$t_{0.01A} = \ln\left(\frac{(0.01)^{1-m}-1}{b}\right)\cdot\frac{1}{-k} \quad \text{for } m > 1 \qquad (4)$$

$$t_{0.01A} = \ln \frac{1 - (0.01)^{1-m}}{b} \cdot \frac{1}{-k} \quad \text{for } m < 1 \qquad (5)$$

where $t_{0.01A}$ was in terms of days after the onset of imbibition.

In order to facilitate and simplify treatment comparison, Lehle and Putnam (1982) had incorporated the onset of 1% germination ($t_{0.01A}$), the weighted mean germination rate (R), and the final cumulative germination percentage (A) into a single numerical index (I) defined as:

$$I = \frac{AR}{t_{0.01A}} \qquad (6)$$

The reduction of the index value by 50% (I_{50}) were estimated from linear calibration of extract concentration versus index values for each replicate and treatment.

An illustration which shows that extremes of germination pattern differences can be defined in terms of these germination aspects is shown in Figure 2. Additionally, the study by Lehle and Putman (1982) showed that Richards' function produced better fits of more reasonable biological forms than did a polynomial of equal parameter number (Figure 3).

Statistical analysis At first, a split-split plot design with two replications was used for each experiment. The main plot was the soil tillage practices, namely: no-till, disc, plot, and control. The split-plot was the different number of days the resin column was attached to the circulating mechanism, namely: 1, 3, and 6 days. The split-

Parameter	Curve A	B	C	D
Max. germination, %	86	57	86	86
Germination onset, days	1.6	1.6	1.6	2.2
Germination rate, %·days^{-1}	84	84	54	84
Germination index, (%·days^{-1})^2	4520	2990	2900	3280

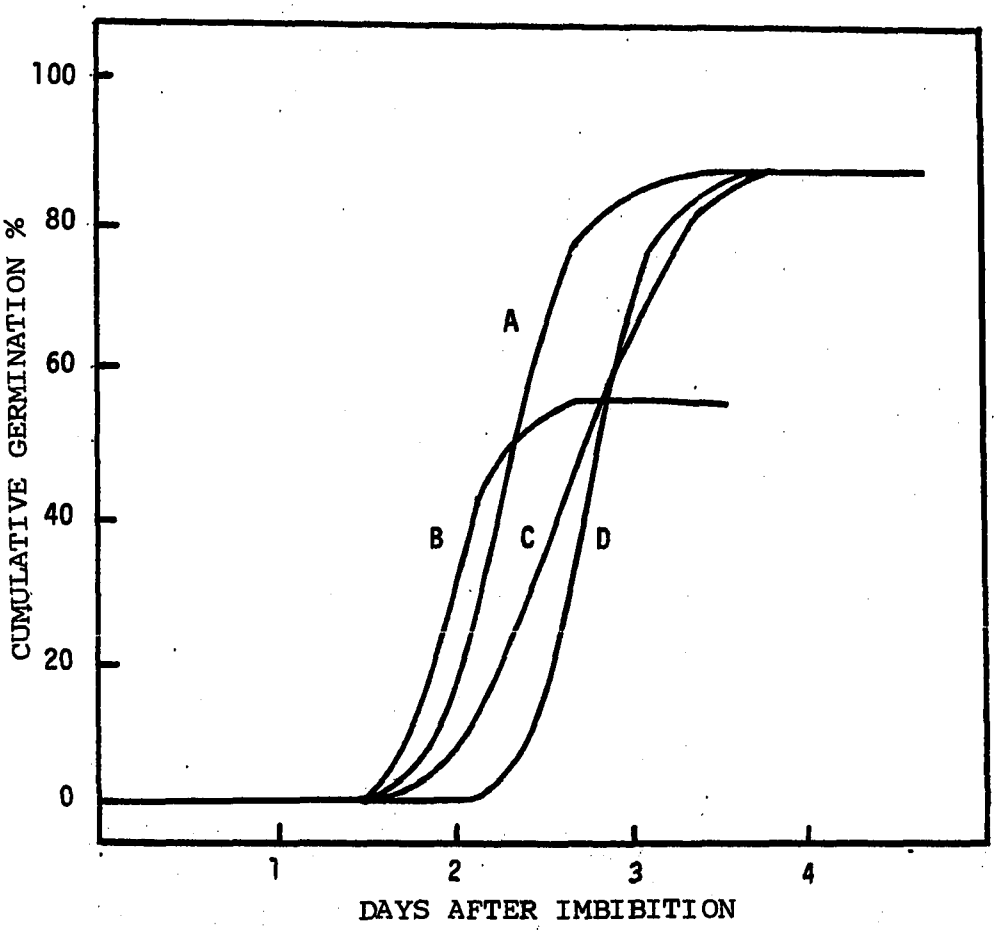

Figure 2. Hypothetical germination curves generated from Richards' function illustrating that the extremes of germination pattern differences can be defined in terms of germination aspects of maximum germination percentage achieved, germination rate, germination onset and germination index (after Lehle and Putnam, 1982)

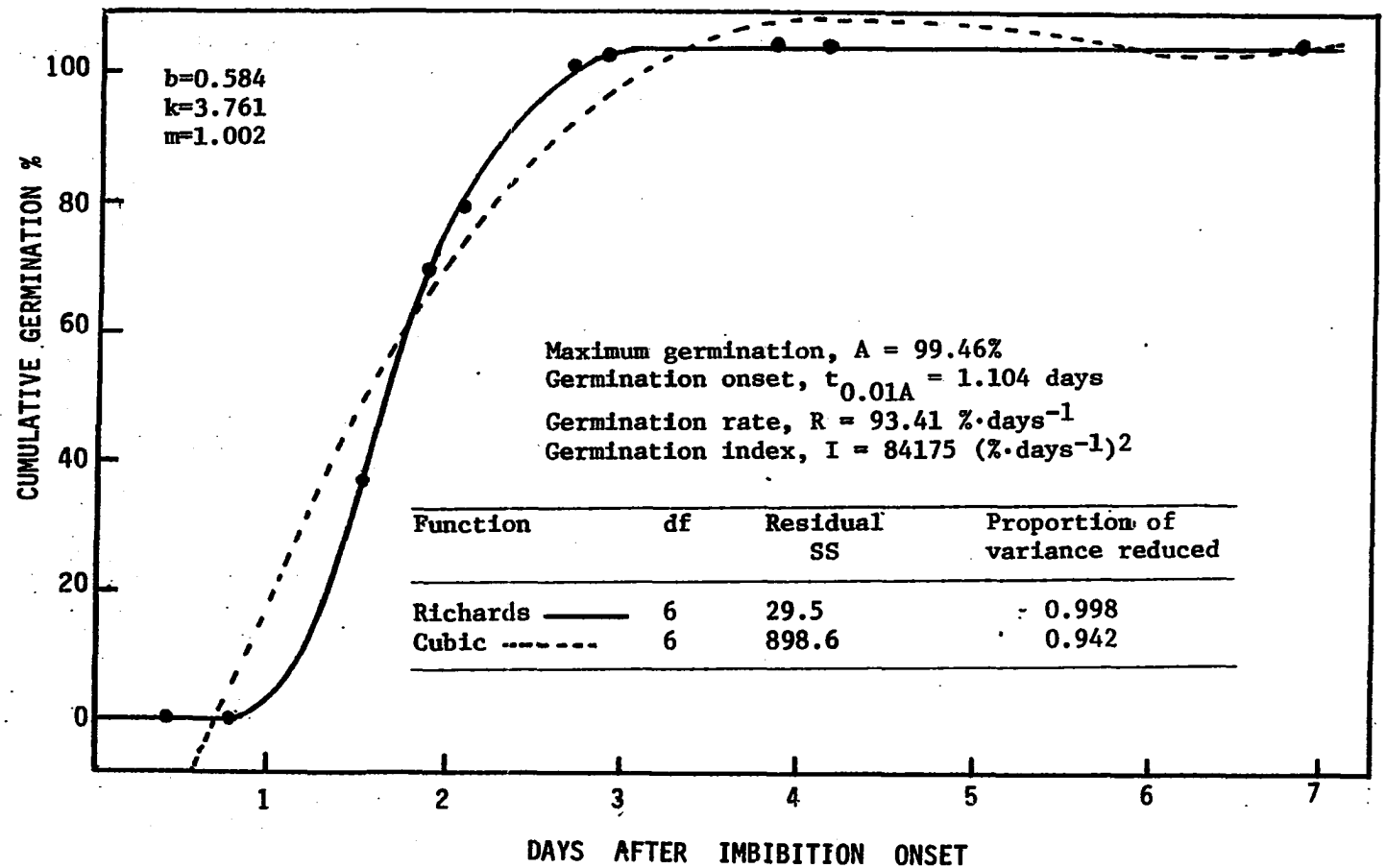

Figure 3. Comparison of curve fitting by Richards' function and the cubic polynomial for cumulative germination of 100 cress seeds imbibed in water at 25°C in the dark (after Lehle and Putnam, 1982)

split-plot was the concentrations of each extract which were 0, 0.5, 1.0, and 2.0 ml.

However, it was later observed that it was better just to use the one-day resin column in the analysis since most of the allelopathic substances were already extracted by this time. The experimental design that was used then was a split-plot design. The main plot was the soil tillage practices and the split-plot was the extract concentrations.

In order to determine the monthly variations of the different tillage practices, the onset of 1% germination ($t_{0.01A}$), the weighted mean germination rate (R), the final cumulative germination percentage (A), the germination index (I), and the extract concentration which gives a 50% reduction (I_{50}) were all expressed as a percentage of control.

The $LSD_{.05}$ given in the figures for Experiments 3 to 8 and Experiments 10 to 15 is for the comparison of any tillage treatments at any monthly treatments.

The 1982 growing season at Ames Iowa had a greater than normal spring precipitation (15 cm for May) and a near normal summer precipitation. Summer temperatures were way below normal because of low solar radiation particularly during July and August.

RESULTS

Growth Chamber Experiments

Experiment 1

This experiment showed that there were significant differences in plant height between the different treatments even during the early growth of corn. During the first four weeks of growth, the corn seedling plant height in the corn residue soil treatment was significantly different from the other treatments (Table 1 and Figure 4). Four weeks after planting, the treatment with fallow soil plus coarse corn residue (95 cm) grew faster than the fallow soil (87 cm) and the other treatments. Corn residue soil treatment (78 cm) was the shortest plant height after four weeks.

In terms of fresh and dry root weight, shoot weight, biomass weight, and root/shoot ratio, the corn residue soil treatment was the smallest and was significantly different from the other treatments (Tables 2 and 3, Figures 5 and 6). It also was observed that fallow soil plus coarse corn residue gave stimulatory effect in dry shoot weight (1.40 g) and biomass weight (1.70 g) as compared with the control (1.09 g for shoot weight and 1.38 g for biomass weight).

Table 1. Plant height of corn seedlings at one, two, three, and four weeks after planting[a]

Treatments	Plant height (cm)			
	Week 1	Week 2	Week 3	Week 4
Fallow soil (control)	19a	37a	57ab	87b
Fallow + coarse residue	21a	40a	64a	95a
Fallow + fine residue	20a	37a	56b	86b
Corn residue soil	13b	27b	47c	78c

[a]All means in a column not followed by the same letter are significantly different from one another at 5% level of probability as determined by Duncan's multiple range test, in this and all subsequent tables where applicable.

Table 2. Fresh root weight, shoot weight, biomass weight, and root:shoot rates of corn four weeks after planting

Treatments	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
Fallow soil (control)	4.75a	11.64b	16.39b	0.40ab
Fallow + coarse residue	5.86a	16.14a	22.00a	0.37b
Fallow + fine residue	5.77a	12.80b	18.57b	0.45a
Corn residue soil	1.64b	8.59c	10.23c	0.20c

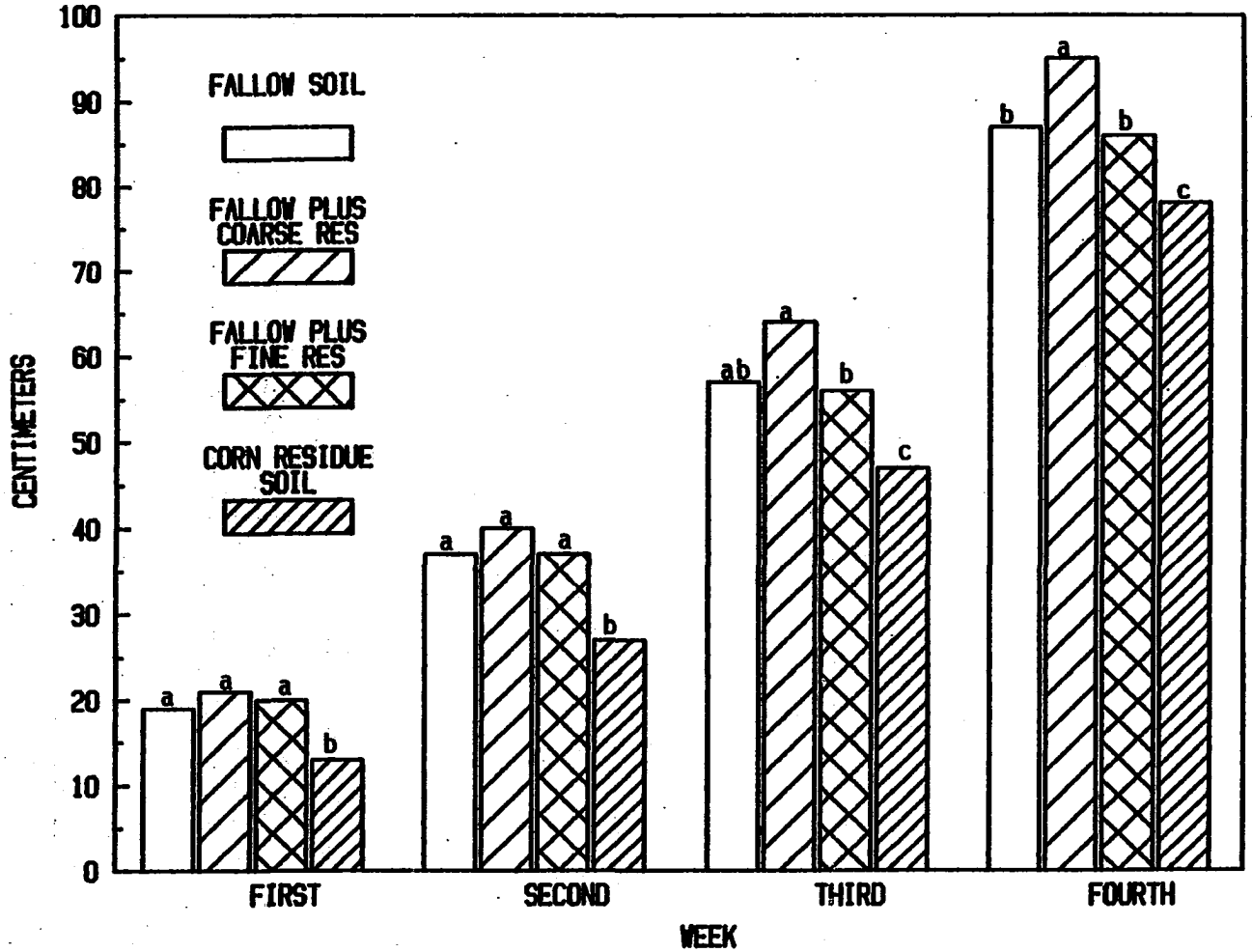

Figure 4. Plant height of corn at one, two, three, and four weeks after planting; bars with different letters within each week are significantly different

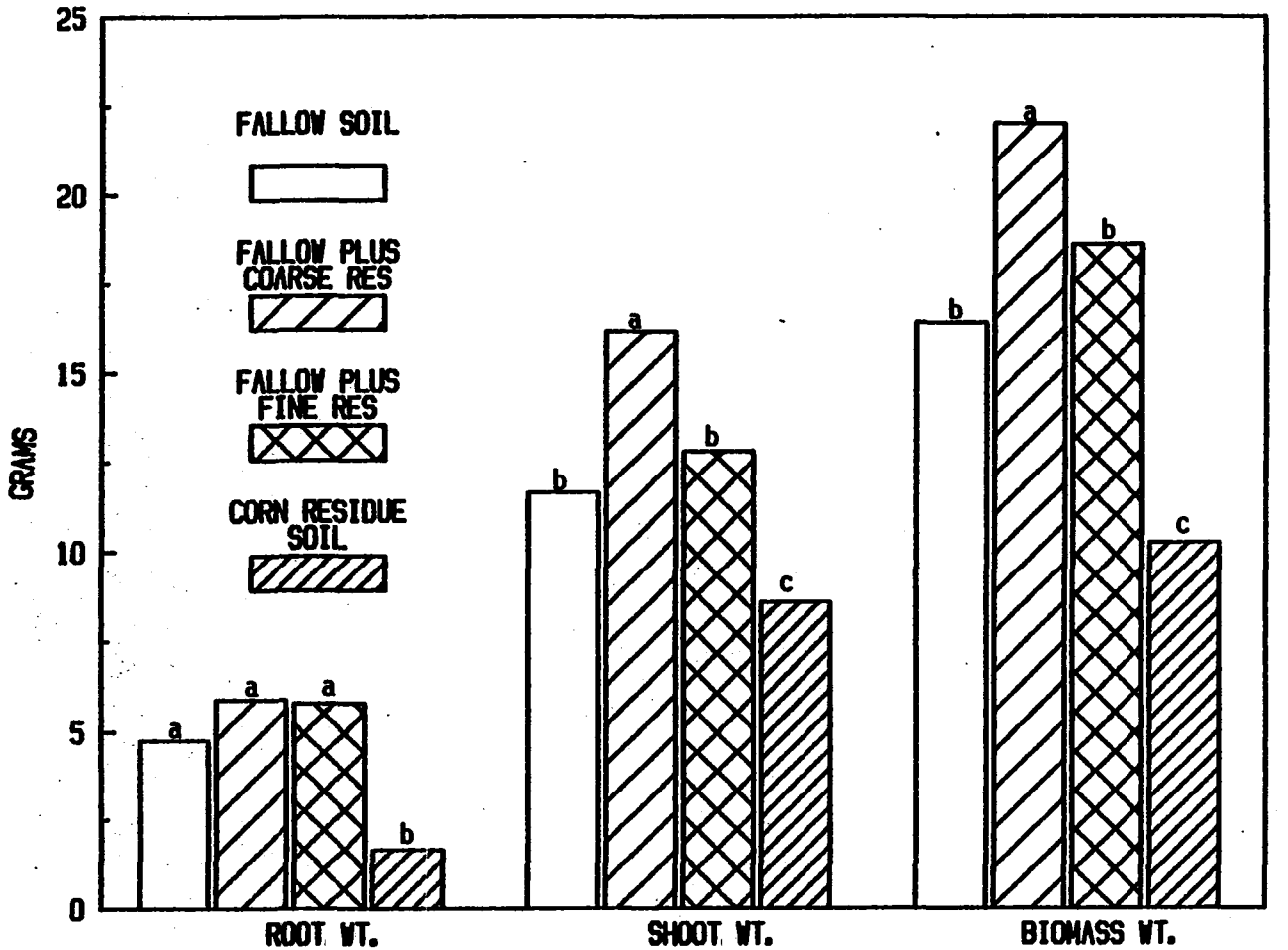

Figure 5. Fresh root, shoot and biomass weights of corn four weeks after planting; bars with different letters in each category are significantly different

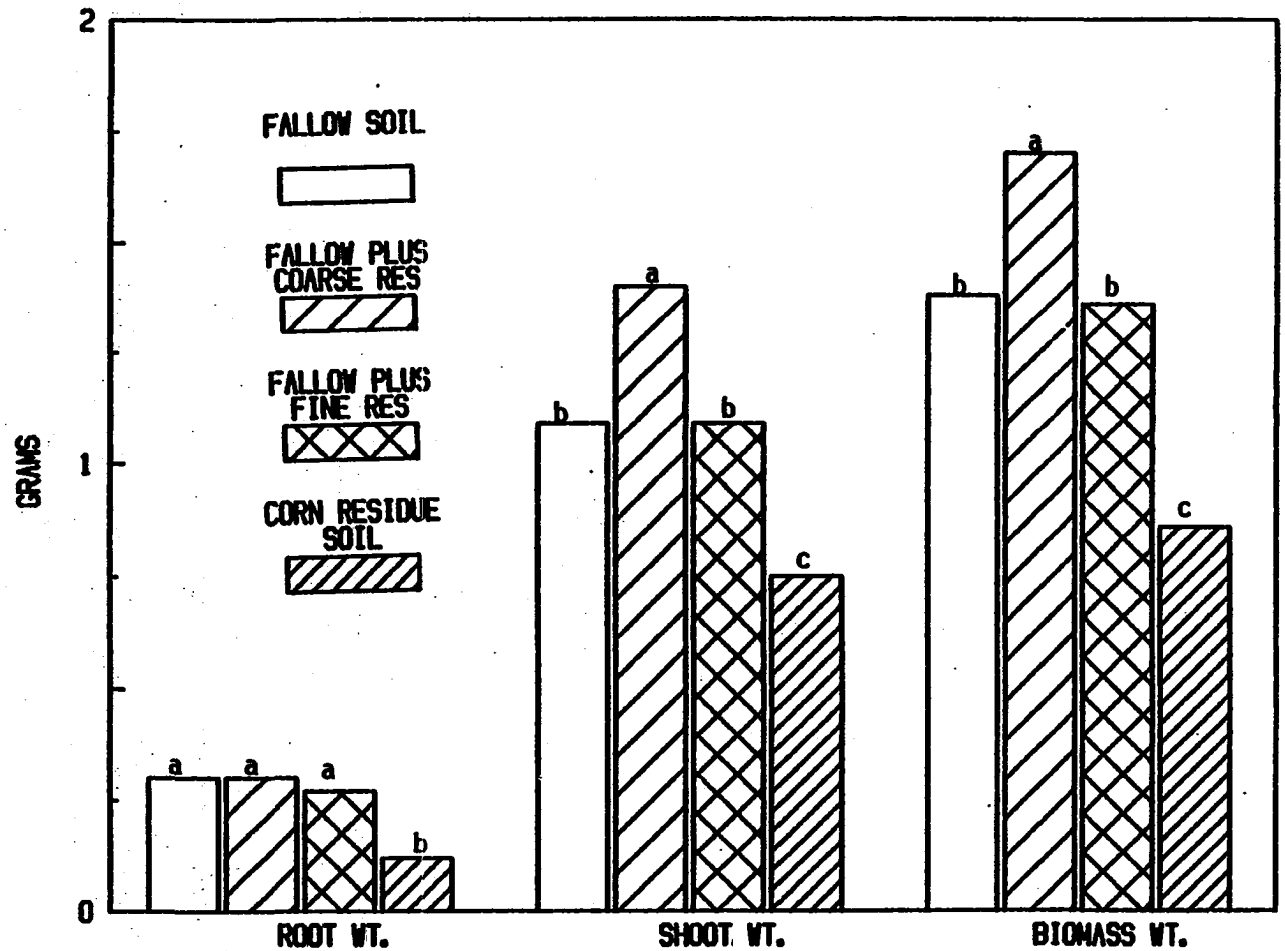

Figure 6. Dry root, shoot and biomass weights of corn four weeks after planting; bars with different letters in each category are significantly different

Table 3. Dry root weight, shoot weight, biomass weight, and root:shoot ratio of corn four weeks after planting

Treatments	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
---	---	---(g)---		
Fallow soil (control)	0.30a	1.09b	1.38b	0.27a
Fallow + coarse residue	0.30a	1.40a	1.70a	0.25a
Fallow + fine residue	0.27a	1.09b	1.36b	0.22a
Corn residue soil	0.12b	0.75c	0.86c	0.16b

Experiment 2

In order to verify whether the difference between the soil without corn residue treatment (control) and the soil with corn residue treatment was due to the allelopathic effect of corn residues and not from nitrogen deficiency, this experiment was performed. A split-plot design was used with soil samples (soil without corn residue and soil with corn residue soil) as the main plot and nitrogen rate (100, 200, and 400 kg N/ha) as the split-plot.

The results indicated that plant height of the soil-without-corn-residue treatment was higher than the soil with corn residue treatment and that significant differences were observed during the first week of growth as shown in Table 4.

It was further observed that the main effect of the corn residue was on root weight. Fresh and dry root weights in the soil without corn residue treatment were greater than in

Table 4. Plant height of corn seedlings at one, two, three and four weeks after planting using soils with corn residue and soils without corn residue

Treatments	Plant height (cm)			
	Week 1	Week 2	Week 3	Week 4
- corn residue	9a	39a	50a	92a
+ corn residue	6b	36a	50a	89a

the soil with corn residue treatment (Figures 7 and 8). The fresh root weight of the soil-without-corn-residue treatment and the soil-with-corn-residue treatment were 6.12 and 3.61 g, respectively. The dry root weight of the soil-without-corn-residue treatment and the soil-with-corn-residue treatment were 0.55 and 0.33 g, respectively. Additionally, significant differences in root:shoot ratio was also observed between these two treatments (Tables 5 and 6).

Table 5. Fresh root weight, shoot weight, biomass weight, and root:shoot ratio of corn seedlings four weeks after planting using soils with corn residue and soils without corn residue

Treatments	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
- corn residue	6.12a	13.25a	19.4a	0.47a
+ corn residue	3.61b	16.79a	20.4a	0.23b

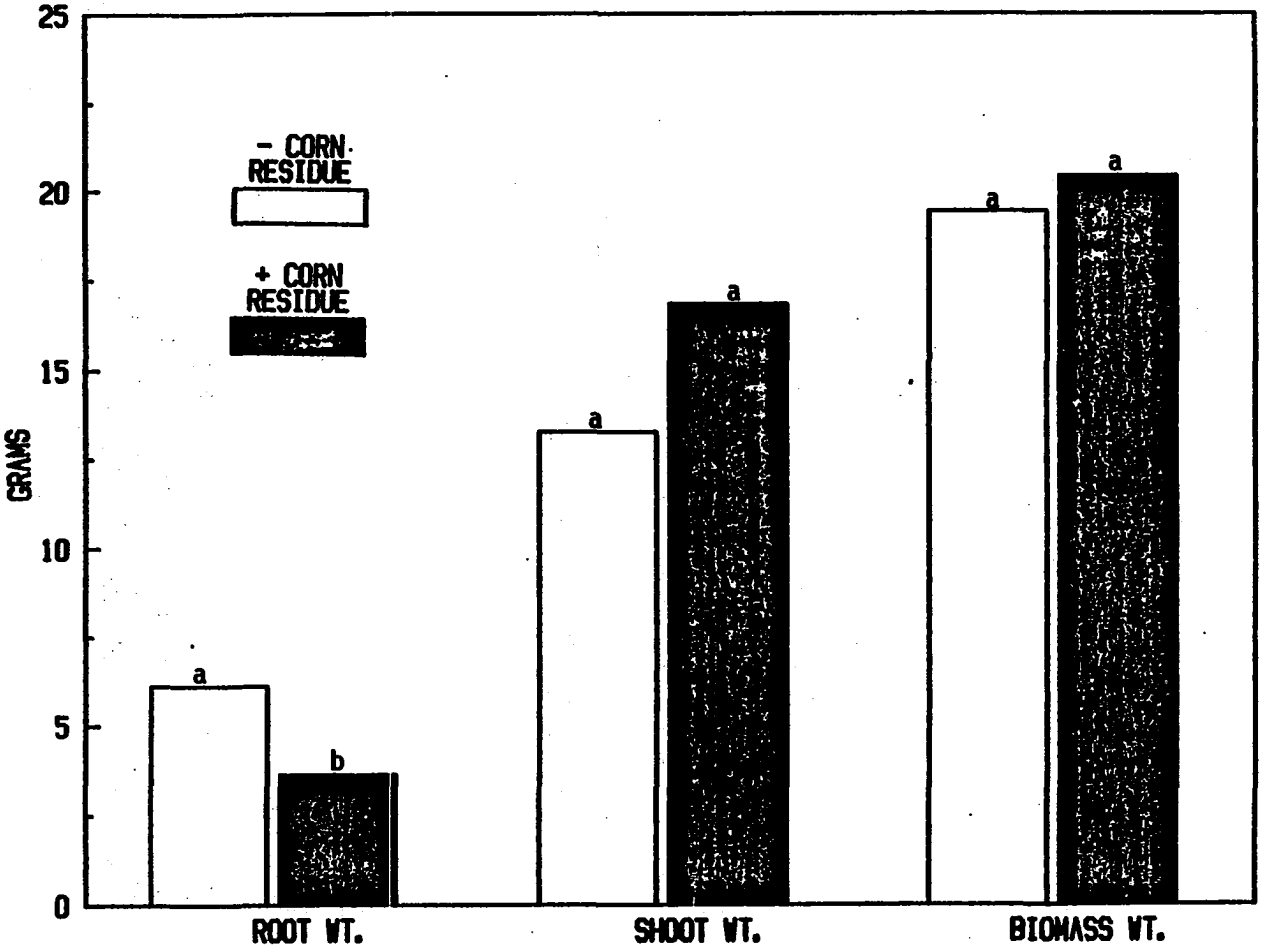

Figure 7. Difference between soils without corn residue and soils with corn residue in terms of dry root, shoot, and biomass weights of corn four weeks after planting; bars with different letters in each category are significantly different

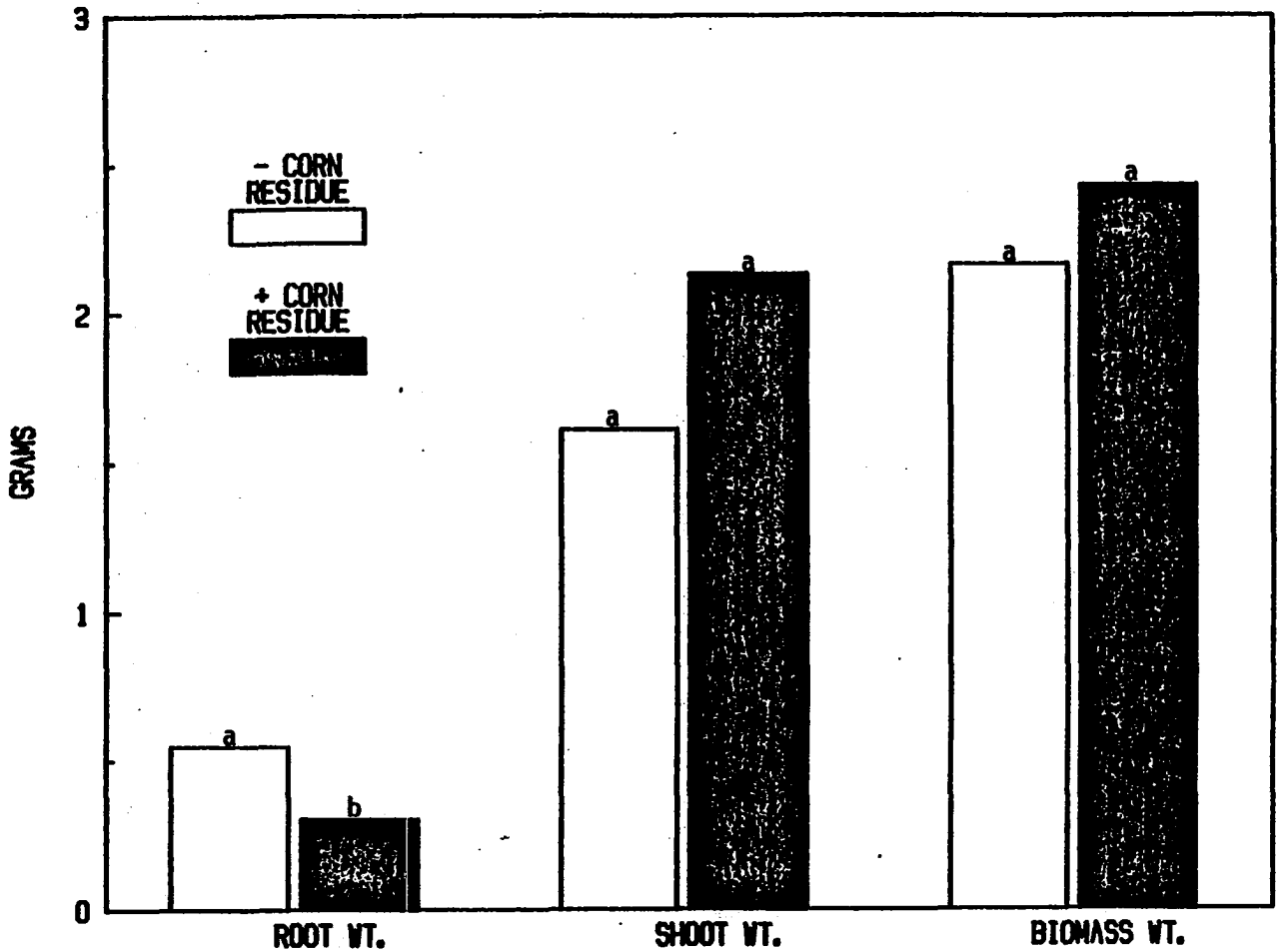

Figure 8. Difference between soils without corn residue and soils with corn residue in terms of fresh root, shoot, and biomass weights of corn four weeks after planting; bars with different letters in each category are significantly different

Table 6. Dry root weight, shoot weight, biomass weight, and root:shoot ratio of corn seedlings four weeks after planting using soils with corn residue and soils without corn residue

Treatment	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
---	---	---(g)---		
- corn residue	0.55a	1.61a	2.16a	0.35a
+ corn residue	0.33b	2.13a	2.43a	0.16b

In contrast, the soil-with-corn-residue treatment tended to have higher fresh and dry shoot weights and biomass weight than the soil-without-corn-residue treatment. However, they were not found to be statistically significantly different from each other.

Looking at the effect of the split-plot, which was the different nitrogen rates (100, 200, and 400 kg N/ha), it was observed that plant height, fresh and dry root weights, biomass weight, and root:shoot ratio were not significantly different from each other (Tables 7, 8, and 9). However, the 200 kg N/ha treatment tended to have a taller plant height.

Experiments 3 to 8

Soil samples collected in April, May, June, July, August, and September 1982 corresponded to Experiments 3, 4, 5, 6, 7, and 8. Results of the experiments using these monthly collected soil samples revealed that there were significant

Table 7. Plant height of corn seedlings one, two, three, and four weeks after planting using different rates of nitrogen

Nitrogen kg/ha	Plant height (cm)			
	Week 1	Week 2	Week 3	Week 4
100	7	37	50	89
200	8	40	52	94
400	7	35	49	88

Table 8. Fresh root weight, shoot weight, biomass weight, and root:shoot ratio of corn four weeks after planting using different rates of nitrogen

Nitrogen kg/ha	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
100	4.46	14.97	18.67	0.33
200	5.00	15.89	20.89	0.34
400	5.13	14.22	20.10	0.39

Table 9. Dry root weight, shoot weight, biomass weight, and root:shoot ratio of corn four weeks after planting using different rates of nitrogen

Nitrogen kg/ha	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
100	0.42	1.72	2.14	0.26
200	0.43	1.86	2.30	0.25
400	0.42	2.02	2.45	0.26

differences in plant height at one, two, three, and four weeks after planting, fresh and dry root weights, shoot weights, biomass weights, and root:shoot ratio for the different treatments (Tables A2, A3, A4, A5, A6, and A7 in the Appendix).

In order to determine the monthly variation in plant height, fresh and dry root weights, shoot weights, and biomass weights for the different treatments (control, no-till, disc, and plow), these parameters were all expressed as a percentage of control. Tables 10, 11, 12, and 13 and Figures 9, 10, 11, and 12 show the corn seedling plant heights (% of control) from April to September 1982 for the different tillage practices. It was observed that the growth chamber plants for the April no-till treatment were yellow up to the time the plants were harvested, which was four weeks after planting. Significant differences in plant height between different treatments were observed as early as one week after planting for the months of May to August soil samples (Table 10 and Figure 9). Four weeks after planting, significant differences between treatments in terms of plant height were observed from April to September (Table 13 and Figure 12). Plant heights for the no-till, disc, and plow treatments for the June sample were 115, 109, and 107%, respectively, which showed a stimulatory effect as compared to the other months. For the plow treatment, an inhibitory effect in terms of plant

Table 10. Plant height of corn seedlings expressed as % of control one week after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100a	100a	100b	100ab	100a	100a
No-till	109a	79ab	132a	150a	67b	109a
Disc	110a	88a	124a	55b	70b	96a
Plow	95a	60b	117ab	143a	90a	96a

Table 11. Plant height of corn seedlings expressed as % of control two weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100a	100a	100c	100ab	100a	100a
No-till	99a	99a	110a	111a	83c	101a
Disc	100a	100a	101bc	92b	81c	99a
Plow	94a	94a	107ab	103ab	91b	99a

Table 12. Plant height of corn seedlings expressed as % of control three weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100a	100ab	100c	100b	100a	100a
No-till	97a	106a	122a	114a	93b	104a
Disc	102a	101a	108b	95c	88b	102a
Plow	96a	94b	110b	96bc	93b	97a

Table 13. Plant height of corn seedlings expressed as % of control four weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100ab	100b	100c	100b	100ab	100ab
No-till	101ab	105a	115a	107a	101a	101a
Disc	104a	105a	109b	100b	96bc	93bc
Plow	95b	96c	107b	100b	94c	88c

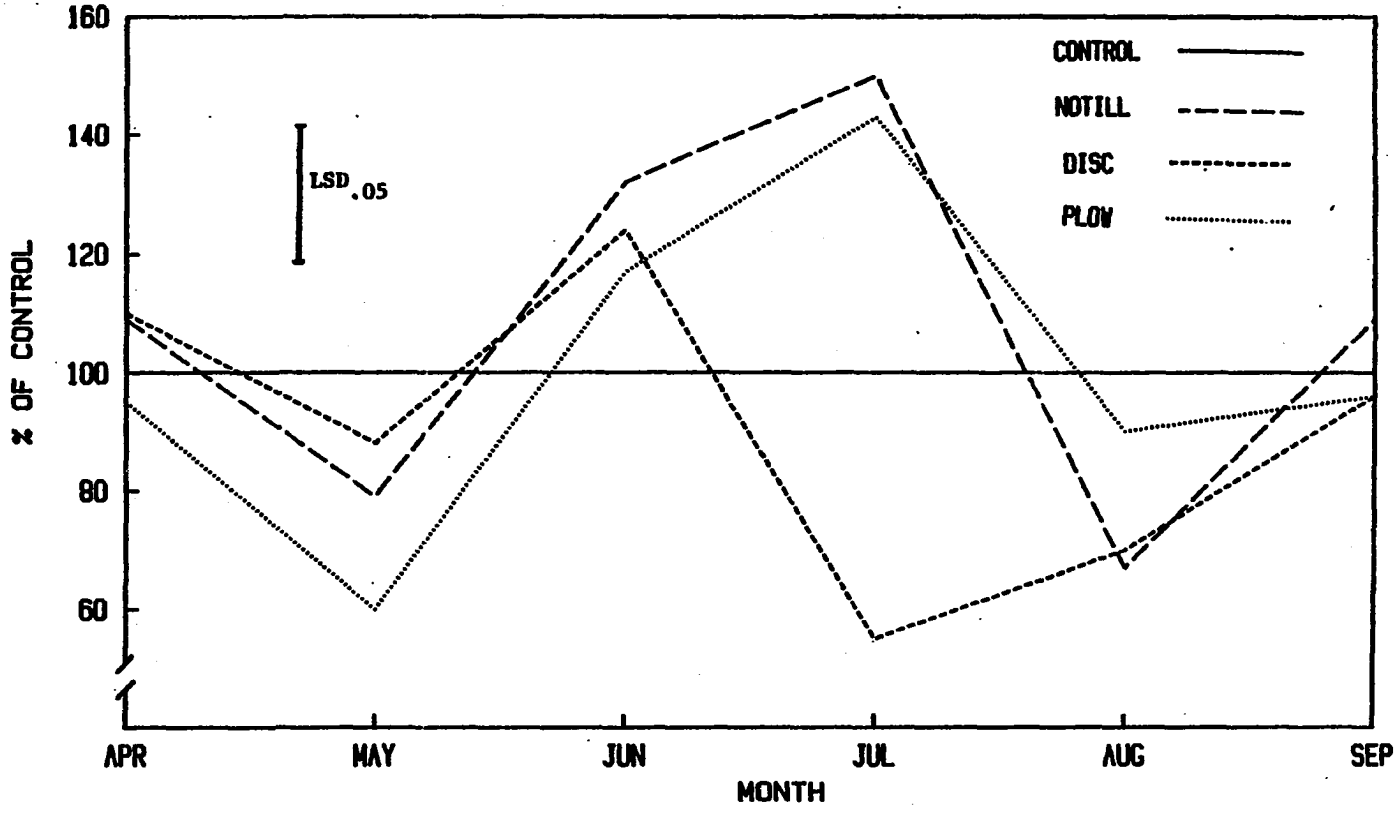

Figure 9. Seasonal variation in corn plant height expressed as % of control one week after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

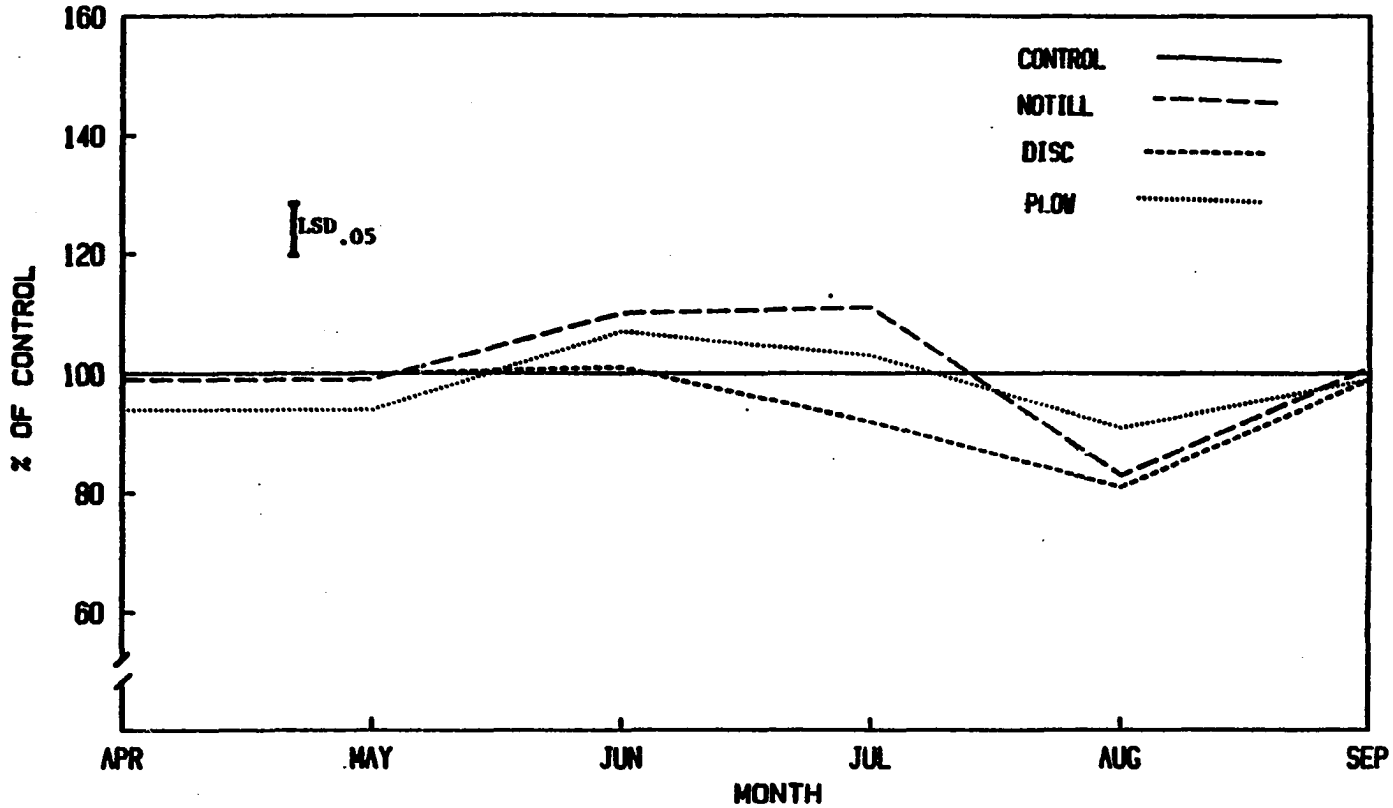

Figure 10. Seasonal variation in corn plant height expressed as % of control two weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

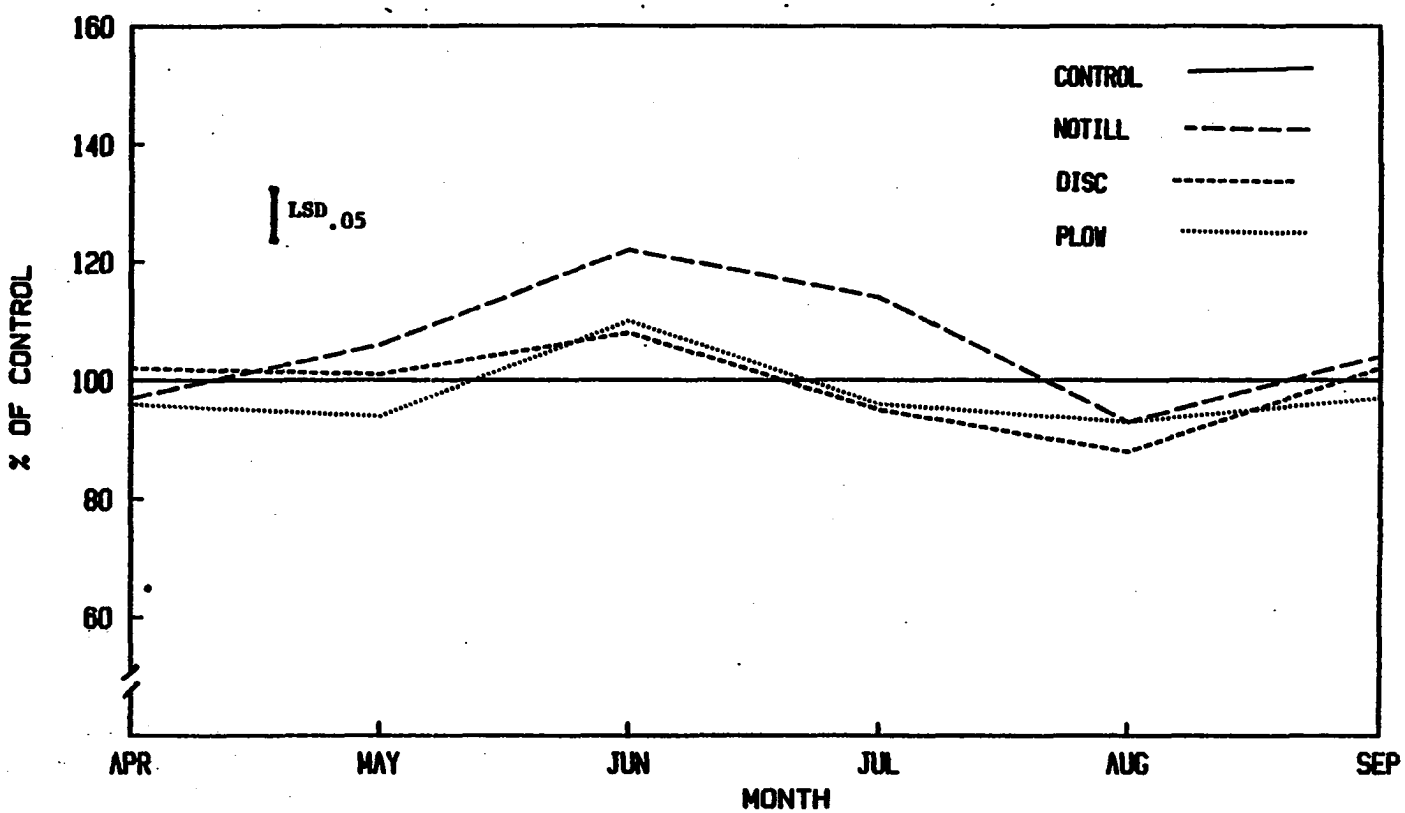

Figure 11. Seasonal variation in corn plant height expressed as % of control three weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

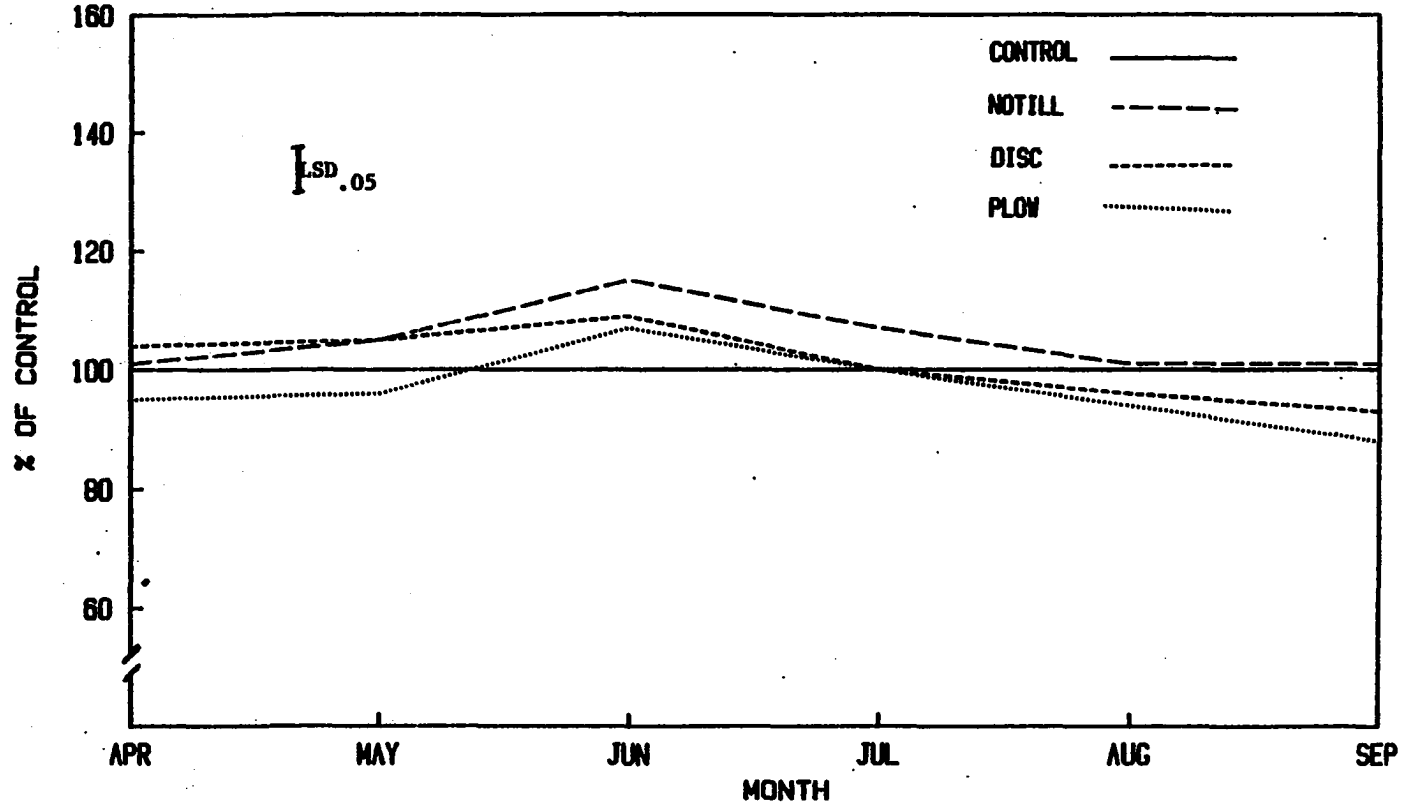

Figure 12. Seasonal variation in corn plant height expressed as % of control four weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

height was observed during the months of April, May, August, and September (95, 96, 94, and 88%, respectively), as shown in Table 13.

Significant differences between the different treatments in terms of fresh and dry shoot weights were observed for the different months (Tables 14 and 15). Fresh and dry shoot weights for the month of June showed stimulatory response for the different tillages as compared with the control. This is illustrated in Figures 13 and 14. The plow treatment showed inhibitory response for the months of April, May, August, and September.

It was observed for all months that the fresh and dry root weights of the different treatments were significantly different (Tables 16 and 17). Fresh and dry root weights of the different tillages for the months of April and September showed inhibitory response as compared with the control (Figures 15 and 16). In terms of dry root weight, the no-till treatment showed the most inhibitory response, followed by plow, then disc treatments, during the month of April (Figure 16). However, the no-till treatment gave the highest stimulatory response during the months of June and July. The plow and disc treatments showed the most inhibitory response during the month of August. For the month of September, plow treatment was most inhibiting.

For the fresh and dry biomass weights, significant

Table 14. Fresh shoot weight of corn expressed as % of control four weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100b	100b	100c	100bc	100b	100b
No-till	92b	123a	170a	156a	118a	116a
Disc	119a	126a	129b	111b	86c	92bc
Plow	74c	85c	117b	99c	75c	82c

Table 15. Dry shoot weight of corn expressed as % of control four weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100b	100b	100c	100b	100a	100b
No-till	89bc	110ab	163a	148a	109a	124a
Disc	125a	132a	125b	107b	51b	75c
Plow	77c	93b	114bc	100b	50b	65c

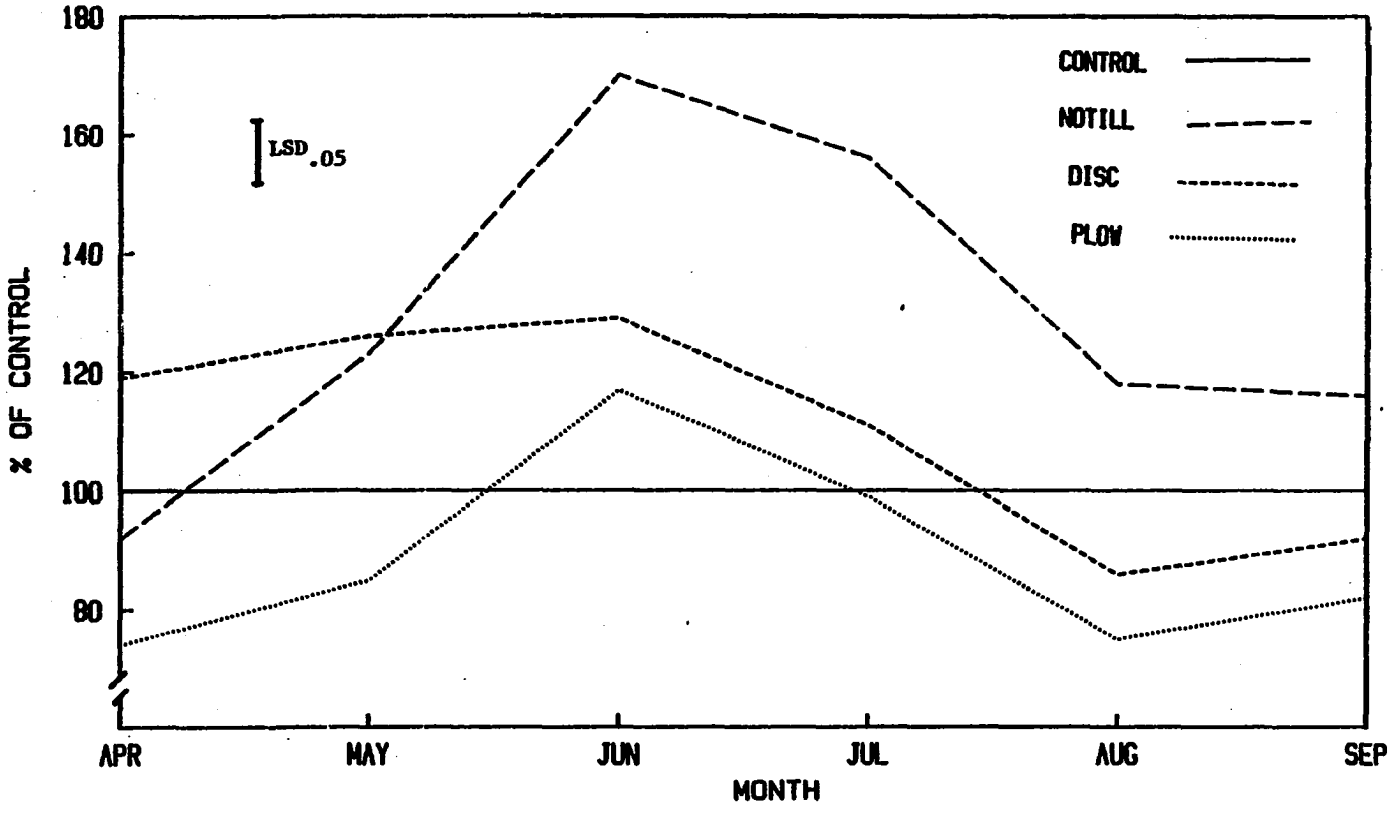

Figure 13. Seasonal variation in corn fresh shoot weight expressed as % of control four weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

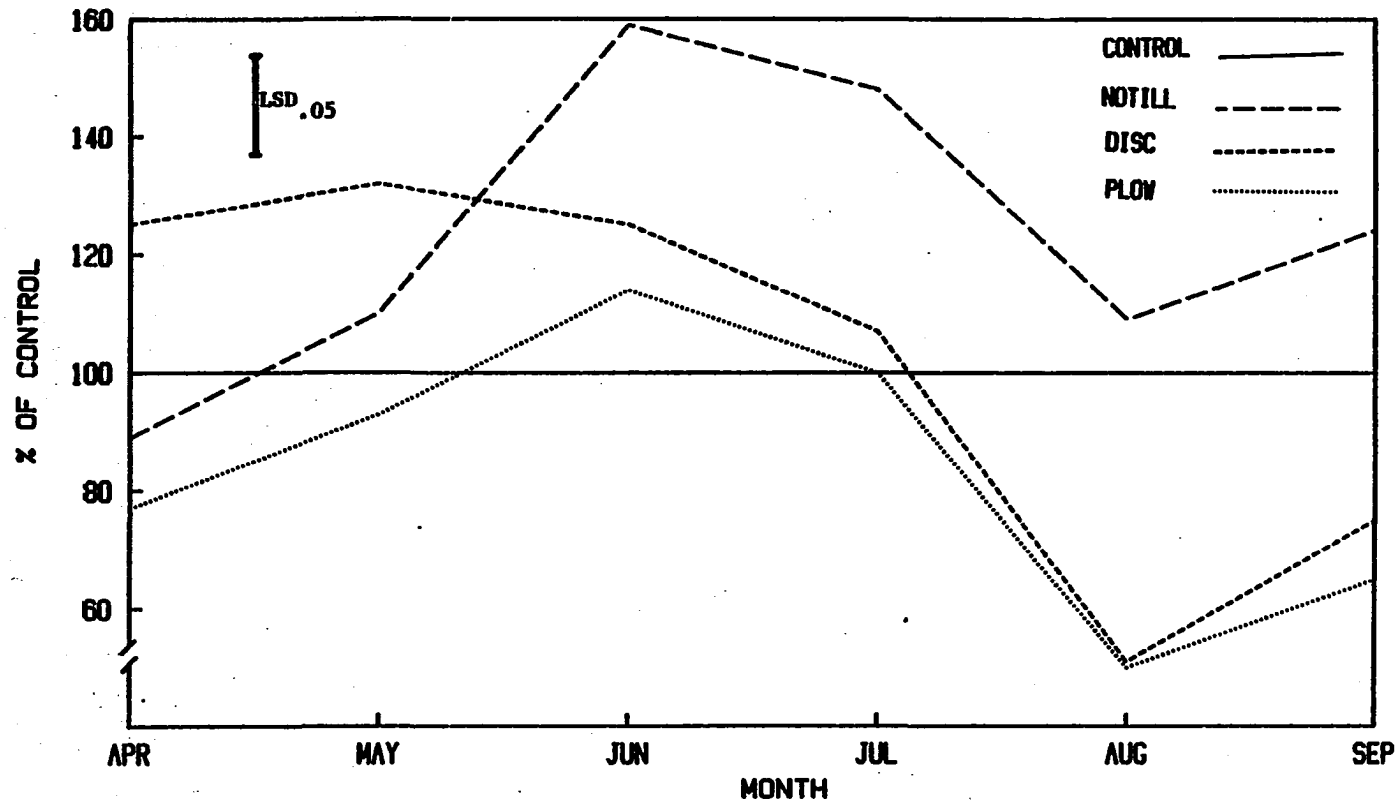

Figure 14. Seasonal variation in corn dry shoot weight expressed as % of control four weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

Table 16. Fresh root weight of corn expressed as % of control four weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100a	100ab	100bc	100b	100b	100a
No-till	67c	107a	128a	122a	113a	85b
Disc	85ab	111a	116ab	108ab	85c	84b
Plow	80bc	90b	96c	109ab	76c	72b

Table 17. Dry root weight of corn expressed as % of control four weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100a	100a	100b	100ab	100a	100a
No-till	70b	95a	124a	113a	98a	85ab
Disc	88ab	100a	112ab	97b	74b	88ab
Plow	80ab	90a	103b	105ab	75b	78b

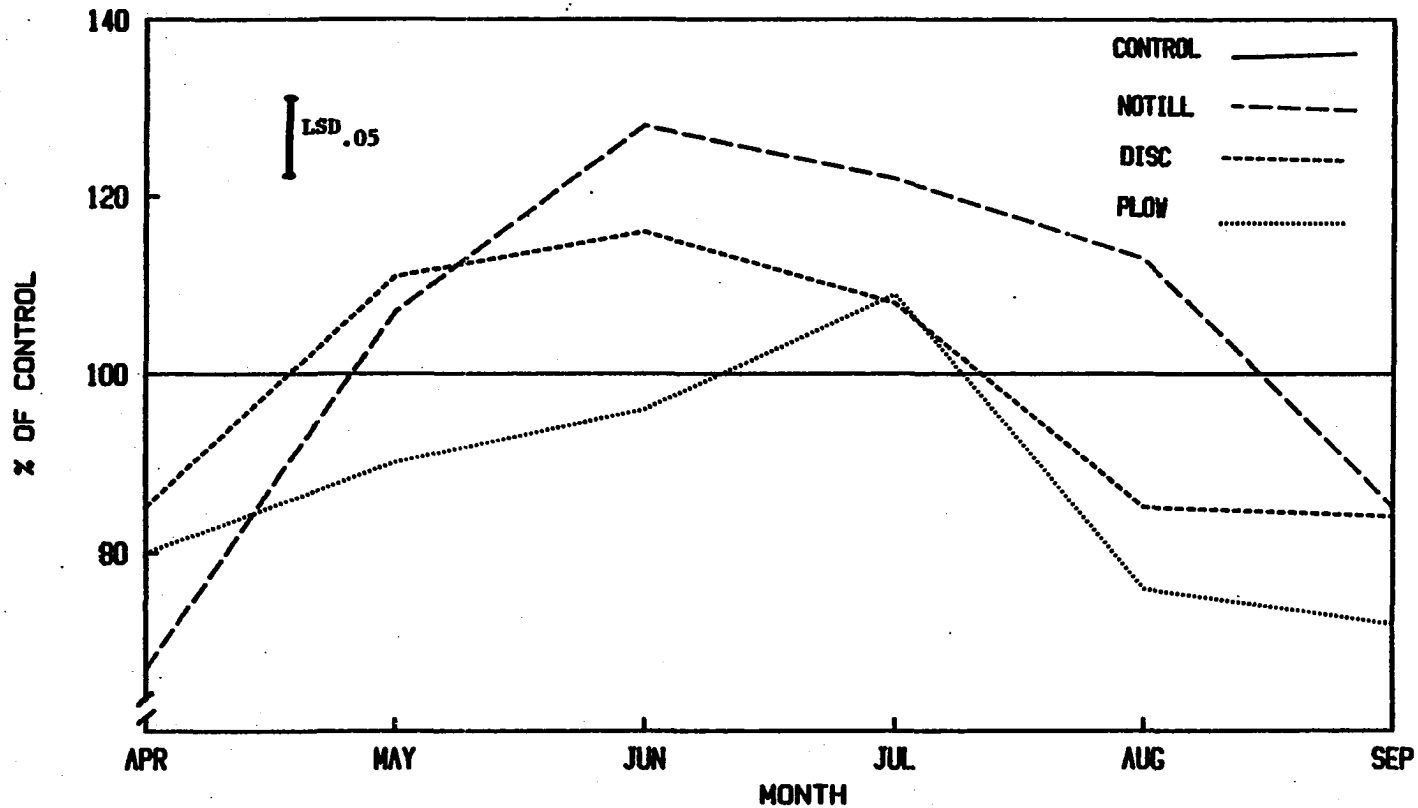

Figure 15. Seasonal variation in corn fresh root weight expressed as % of control four weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

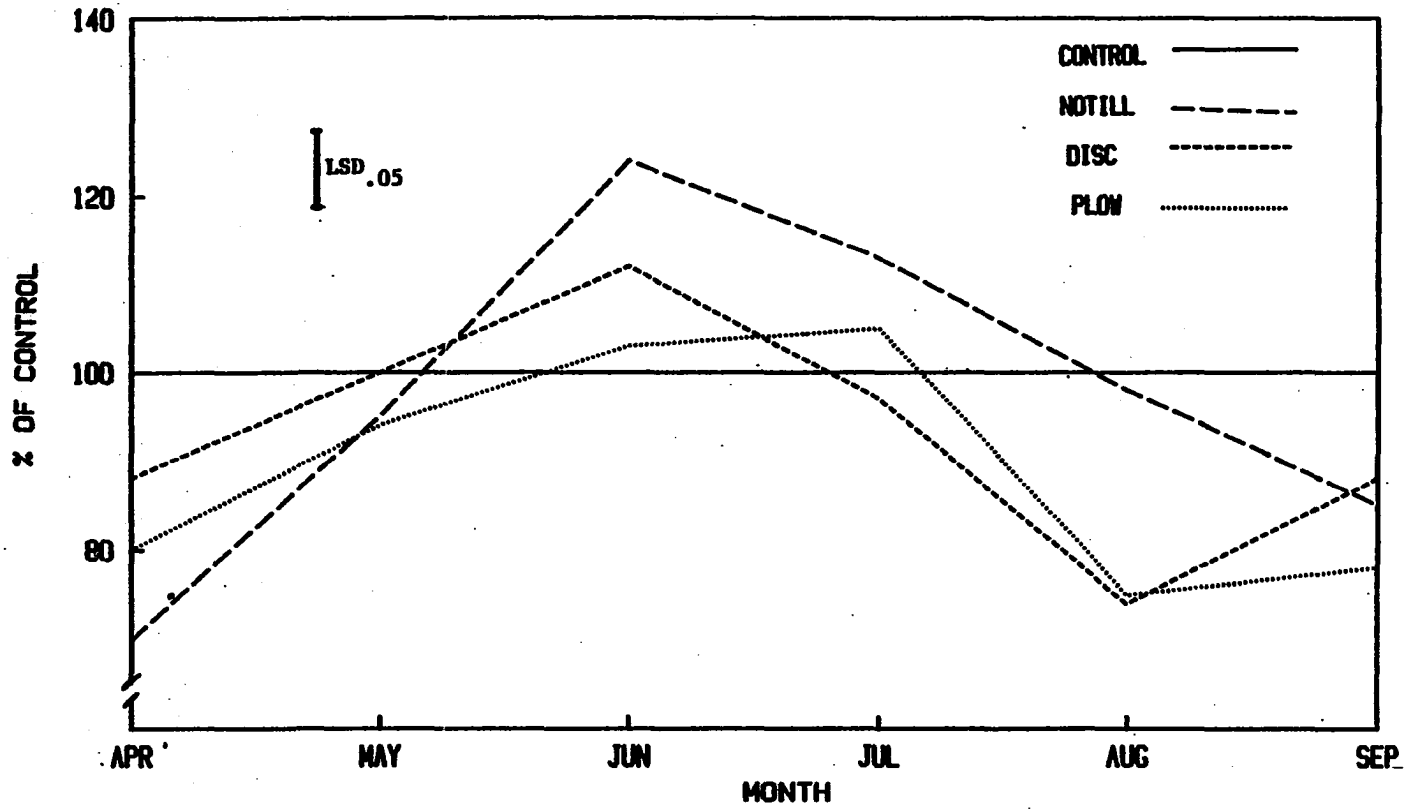

Figure 16. Seasonal variation in corn dry root weight expressed as % of control four weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

differences also were observed between the different treatments for all months, as shown in Tables 18 and 19. Figures 17 and 18 show that the no-till and plow treatments had an inhibitory response during the month of April. However, a stimulatory response was observed for June. The disc treatment which had a stimulatory response during the months of April to June, although not statistically significantly different from the control for April, showed an inhibitory response for the months of August and September which was significantly different from the control. On the other hand, the no-till treatment, which was inhibitory for April, did show a stimulatory response from May to September.

The plant height response for the different months for all the treatments combined, shown in Table 20, reveals that the highest stimulatory response was obtained during the month of June. Figure 19 shows that plant height one and two weeks after planting, as compared with the control, was inhibited during the months of May and August. However, plant height one week after planting had the highest stimulatory response for the month of June.

Fresh and dry root, shoot, and biomass weights for all treatments combined across months are shown in Table 21. Dry root weights during the months of June and July (109 and 104%, respectively) were the highest, while the months of April, August, and September (85, 87, and 89%, respectively) were

Table 18. Fresh biomass weight of corn expressed as % of control four weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100a	100b	100c	100b	100b	100ab
No-till	86b	118a	160a	146a	117a	110a
Disc	110a	122a	126b	110b	85c	93bc
Plow	75b	86c	112c	102b	75c	81c

Table 19. Dry biomass weight of corn expressed as % of control four weeks after planting using April to September 1982 collected soil samples with different tillage practices

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100ab	100b	100c	100b	100c	100b
No-till	85bc	106ab	154a	138a	107a	115a
Disc	116a	122a	122b	105b	55b	78c
Plow	78c	93b	112bc	102b	54b	68c

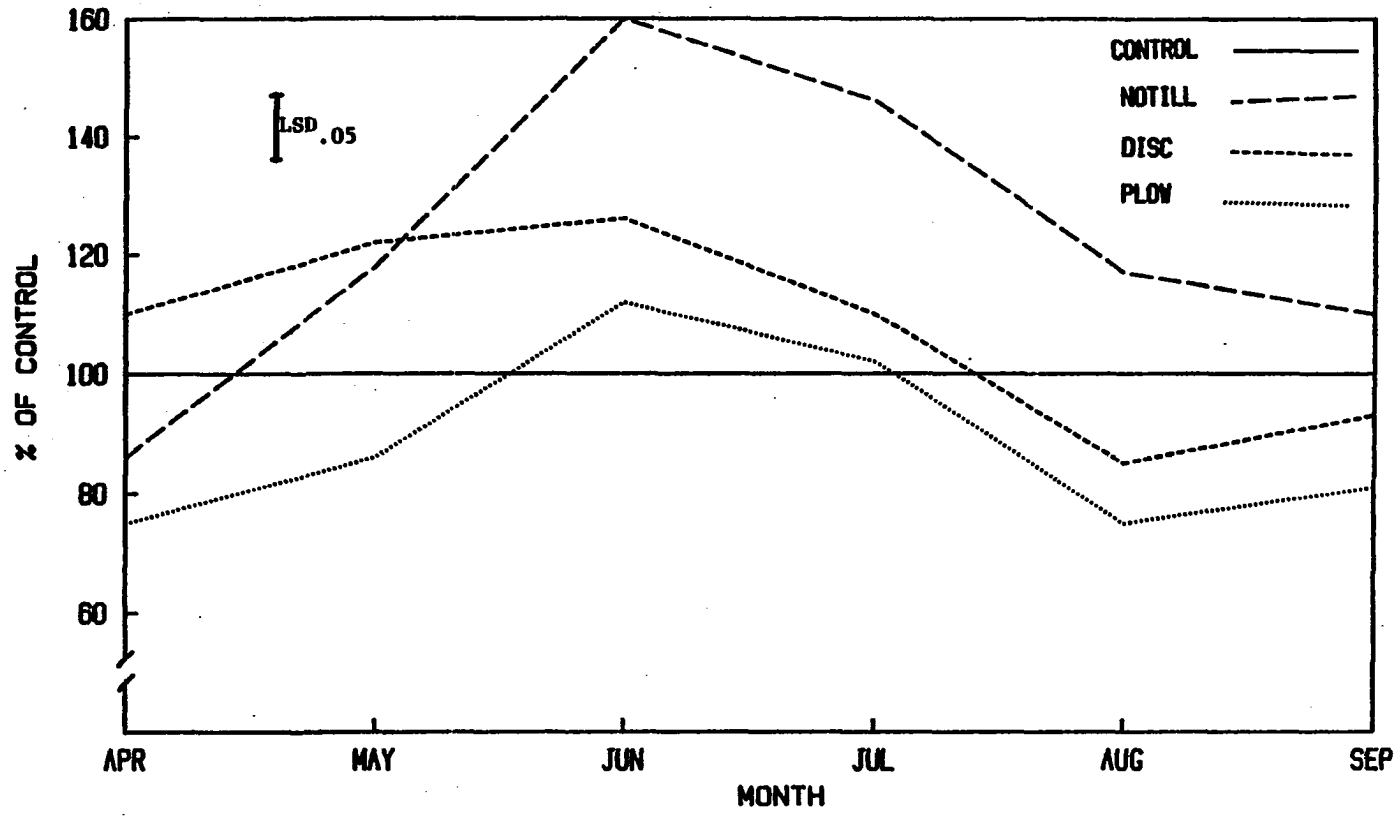

Figure 17. Seasonal variation in corn fresh biomass weight expressed as % of control four weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

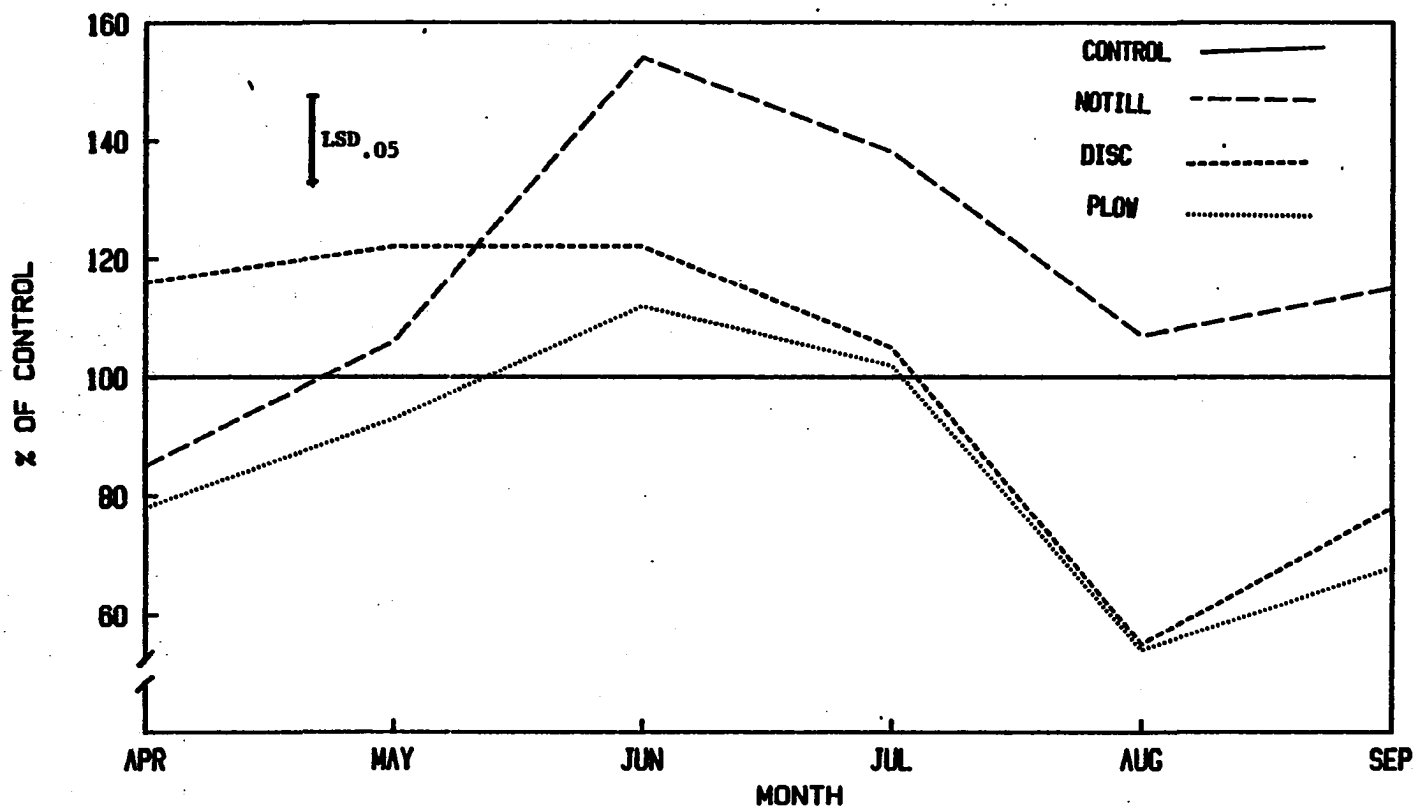

Figure 18. Seasonal variation in corn dry biomass weight expressed as % of control four weeks after planting using soil samples from three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

Table 20. Plant height of corn seedlings expressed as % of control one, two, three, and four weeks after planting using corn residue soils collected from April to September 1982

Month	Plant height			
	Week 1	Week 2	Week 3	Week 4
Apr	104ab	96b	99b	100b
May	82c	77d	100b	101b
Jun	119a	102a	110a	108a
Jul	111ab	100a	101b	102b
Aug	86c	92c	93c	98c
Sep	100b	100a	101b	95d

Table 21. Fresh and dry root, shoot, and biomass weights of corn expressed as % of control four weeks after planting using corn residue soils collected from April to September 1982

Month	Root		Shoot		Total biomass	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Apr	83d	85c	96c	98c	93c	95c
May	102b	98b	108b	109b	107b	105b
Jun	109a	109a	105b	125a	106b	121a
Jul	110a	104ab	116a	114b	114a	111b
Aug	94c	87c	95c	74d	94c	79d
Sep	85d	89c	97c	90c	96c	90c

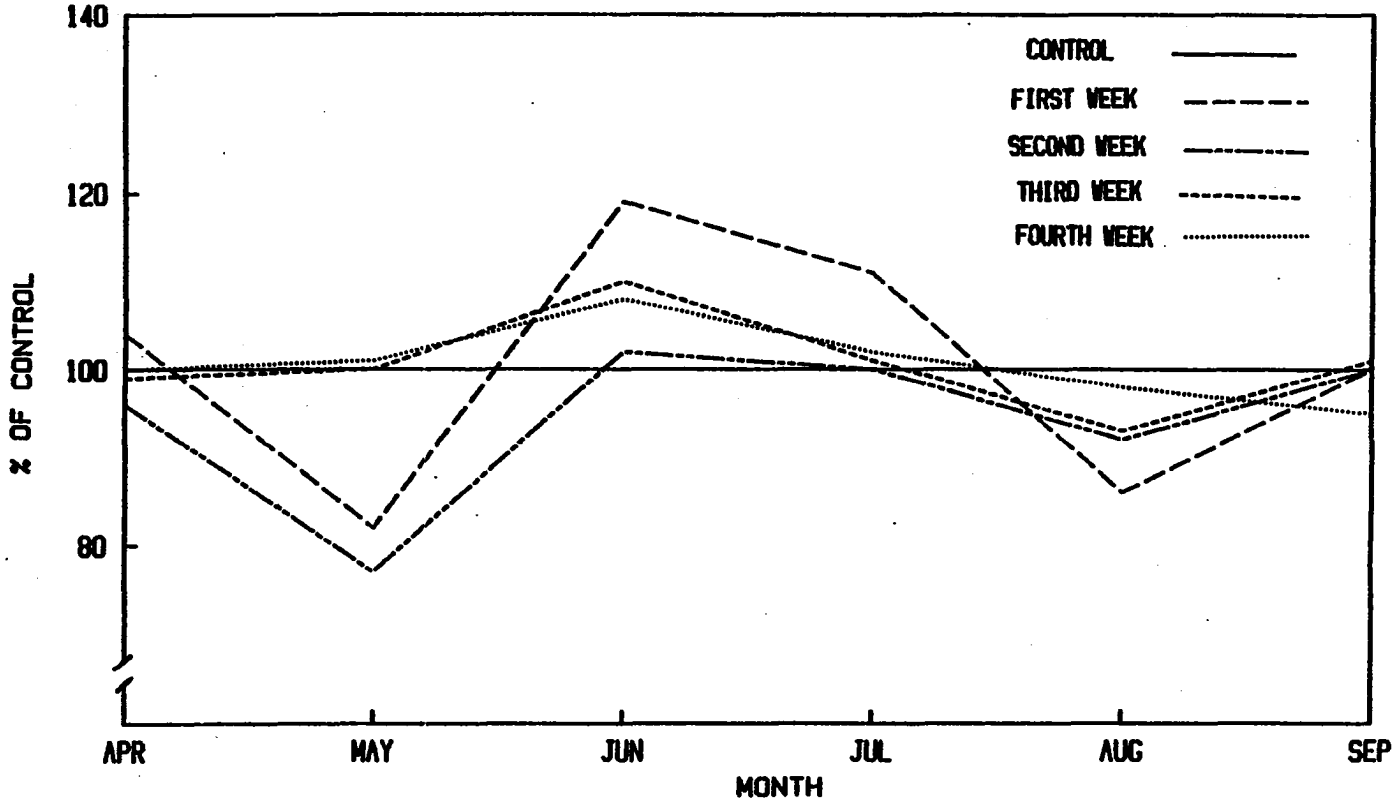

Figure 19. Seasonal variation in corn plant height expressed as % of control at one, two, three, and four weeks after planting using soil samples previously planted to corn collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

the lowest. Dry shoot and biomass weights (125 and 121%, respectively) were highest during the month of June, while the lowest were observed during the months of April (98% for shoot weight and 95% for biomass weight) and September (90% for both shoot weight and biomass weight).

Figures 19 to 22 show the seasonal variation in plant height, fresh and dry root, shoot, and biomass weights. The most pronounced effect was reflected in the fresh and dry root weights. Figure 21 shows that root weight was inhibited during the months of April, August, and September. However, a stimulatory response in root weight was observed during the months of June and July.

Except for the month of August, the root:shoot ratios (by dry weight) of the control and plow treatments for the different months were not significantly different (Appendix Tables A2c to A7c). This means that the rate of root growth is proportional to the rate of shoot growth for these two treatments, although the absolute root and shoot weights of the control treatment were higher than those of the plow treatments. The no-till treatment had a lower root:shoot ratio than the control for all months. The disc treatment had a lower root:shoot ratio for all months except August and September. This means that, for the no-till and disc treatments, the root growth is more inhibited than the shoot growth or that the shoot grew faster than the roots.

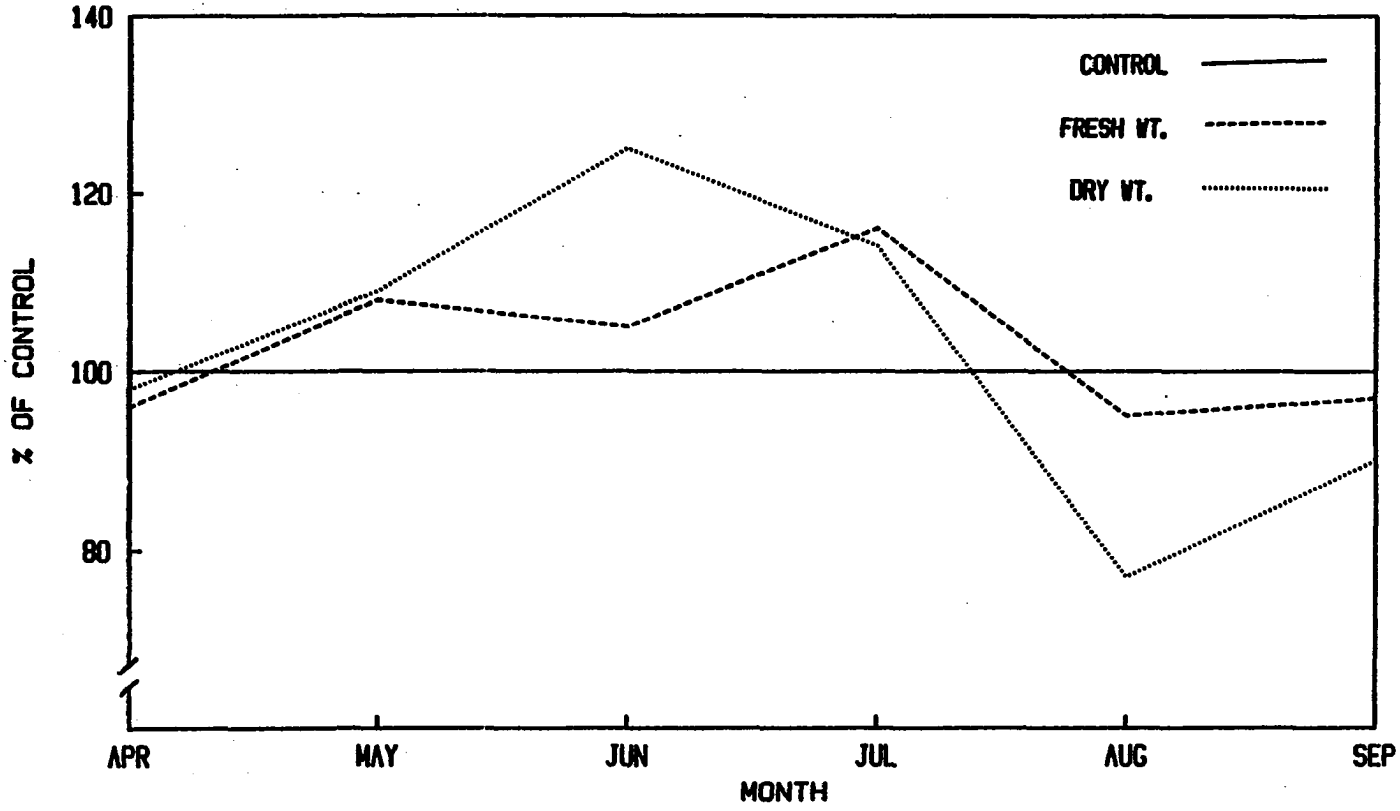

Figure 20. Seasonal variation in corn fresh and dry shoot weights expressed as % of control four weeks after planting using soil samples previously planted to corn collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

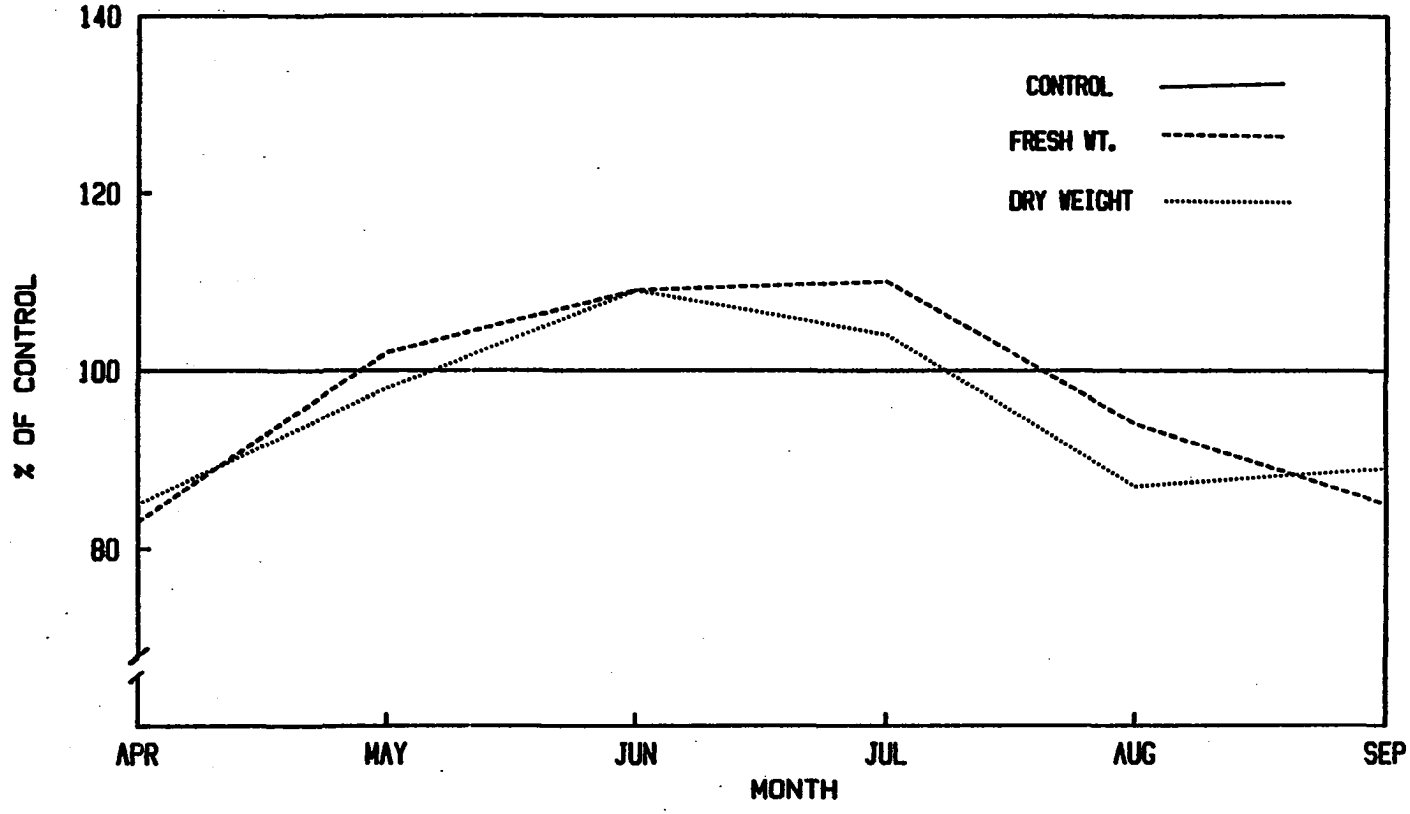

Table 21. Seasonal variation in corn fresh and dry root weights expressed as % of control four weeks after planting using soil samples previously planted to corn collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

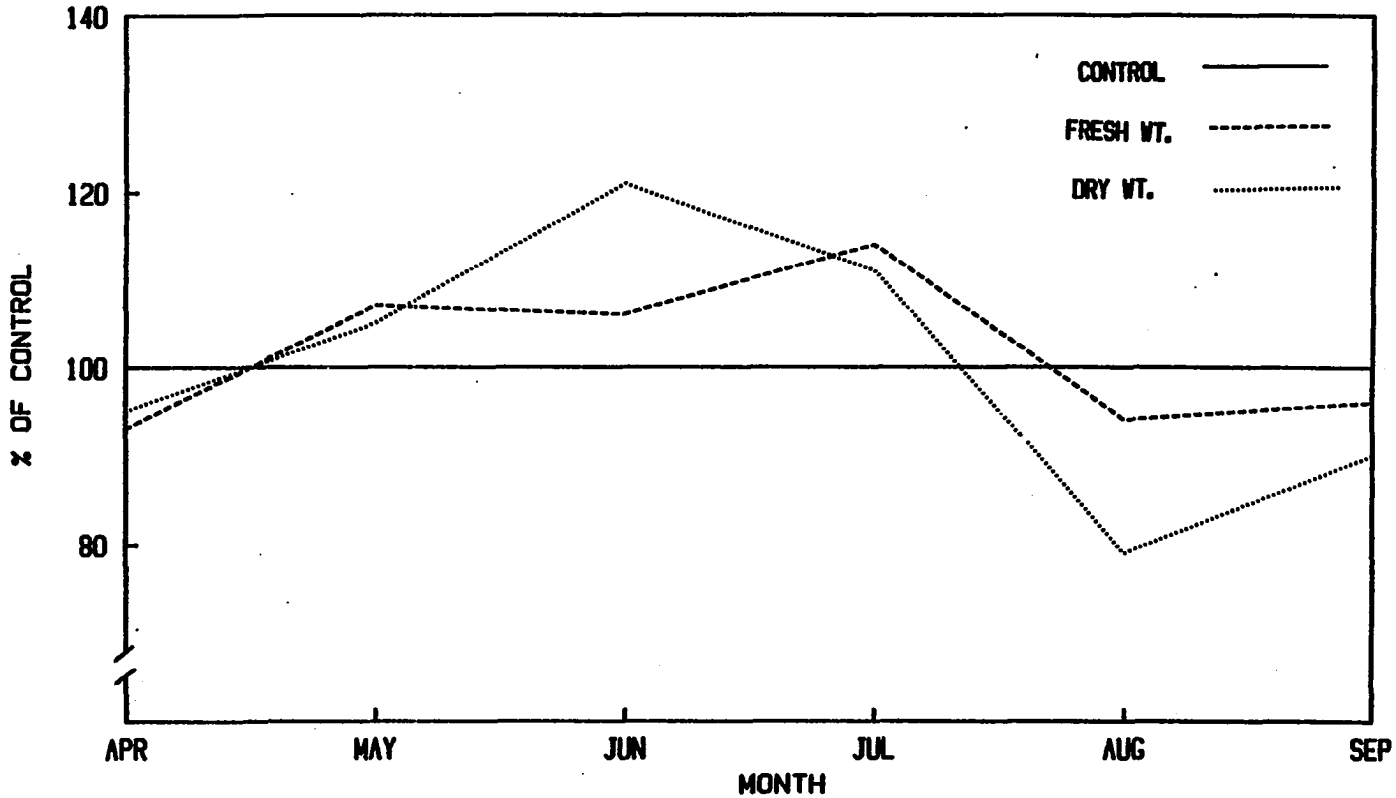

Figure 22. Seasonal variation in corn fresh and dry biomass weights expressed as % of control four weeks after planting using soil samples previously planted to corn collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

Experiment 9

The result of this experiment revealed that the different treatments showed significant differences in plant height at one, two, and three weeks after planting (Table 22). Plant height at one week up to four weeks after planting showed that the control treatment was the tallest compared with the other treatments. An illustration is shown in Figure 23. Four weeks after planting, the treatments (covered between, covered under, uncovered between, and uncovered under) were able to catch up with the plant height of the control treatment such that no significant differences between treatments were observed at this stage.

Table 22. Plant height of corn seedlings at one, two, three, and four weeks after planting using soil media collected from a corn field

Treatments	Plant height (cm)			
	Week 1	Week 2	Week 3	Week 4
Control	6.5a	34a	64a	94a
Covered				
Between	5.0bc	30b	61ab	92a
Under	4.6cd	29b	58b	92a
Uncovered				
Between	4.0d	28b	58b	92a
Under	5.5b	31ab	61ab	92a

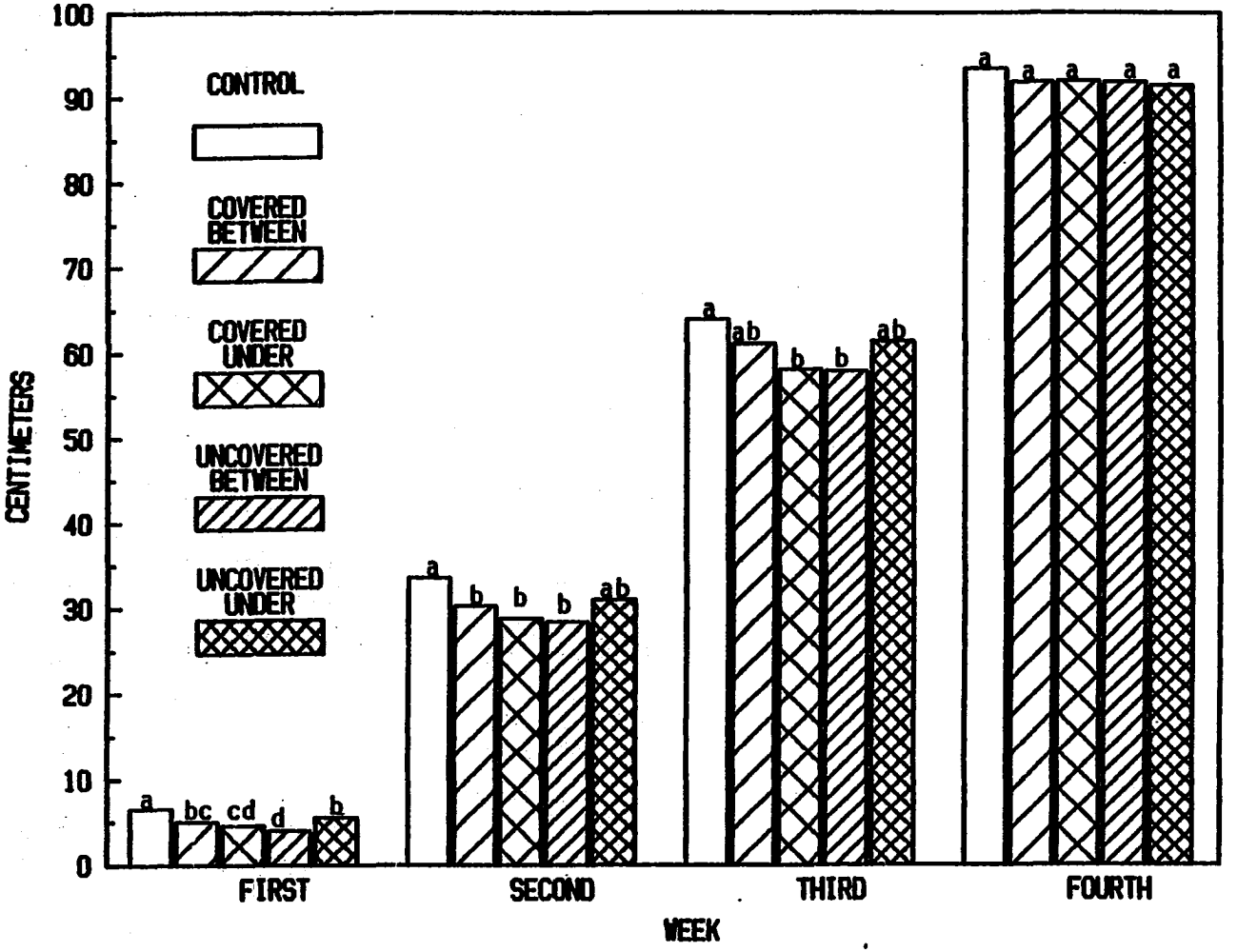

Figure 23. Plant height of corn seedlings one, two, three, and four weeks after planting grown from soils collected from a corn field; bars with different letters in each week are significantly different

The fresh and dry root, shoot, and biomass weights of the different treatments were significantly different. The control always had the highest root, shoot, and biomass weights compared to the other treatments (Table 23, Figures 24 and 25). The dry weights of the root, shoot, and biomass indicated that treatments had an inhibitory effect on corn seedlings compared to the control soil. The dry biomass weights of the covered-between and covered-under treatments (2.88 and 2.82 g, respectively) were significantly different from the control treatment, which was 3.77 g. Moreover, the dry biomass weight of the uncovered-between and uncovered-under treatments (2.32 and 2.30 g, respectively) were significantly lower than the other three treatments. The difference in dry weight between the control and the covered treatments could be due to an accumulation of inhibitory corn root exudates in the soil. Additionally, the difference between the covered treatments and the uncovered treatments could be due to rain-leached substances and/or senescing above-ground plant parts. These results indicated that allelopathic substances originate from senescing above-ground plant parts, rain-leached substances, and root exudates.

Resin Column Experiments

Using a split-split plot design for each experiment, the analysis of variance for the germination index (I), onset of

Table 23. Fresh and dry root, shoot, and biomass weights of corn four weeks after planting using soil media collected from a corn field

	Fresh weight (g)			Dry weight (g)		
Treatments	Root	Shoot	Biomass	Root	Shoot	Biomass
Control	6.52a	15.57a	22.09a	0.81a	2.96a	3.77a
Covered						
Between	5.73a	15.26ab	20.98ab	0.70ab	2.18b	2.88b
Under	4.82b	15.06ab	19.89abc	0.54c	2.27b	2.82b
Uncovered						
Between	4.84b	14.88ab	19.17bc	0.55c	1.77c	2.32c
Under	4.83b	13.72b	18.55c	0.61bc	1.69c	2.30c

1% germination ($t_{0.01A}$), germination rate (R), and maximum germination (A) is shown in Appendix Tables A8, A9, A10, and A11. The tillage practice (no-till, disc, plow, and control) was the main plot, the number of days the allelopathic substances were collected by the XAD-4 resin column (1, 3, and 6 days) was the split-plot and the extract concentration (0, 0.5, 1.0, and 2.0) was the split-split plot. The results of these experiments showed that germination index and onset of 1% germination of the split-plot (1, 3, and 6 days) were statistically significant (Appendix Tables A8 and A9). It was observed that most of the allelopathic substances in all experiments were trapped by the XAD-4 resin after one day. It was for this reason that the subsequent analysis uses only the one-day resin column. Note that extract concentrations were significant when analyzed as a split-split plot (Appendix Tables A8, A9, A10, and A11).

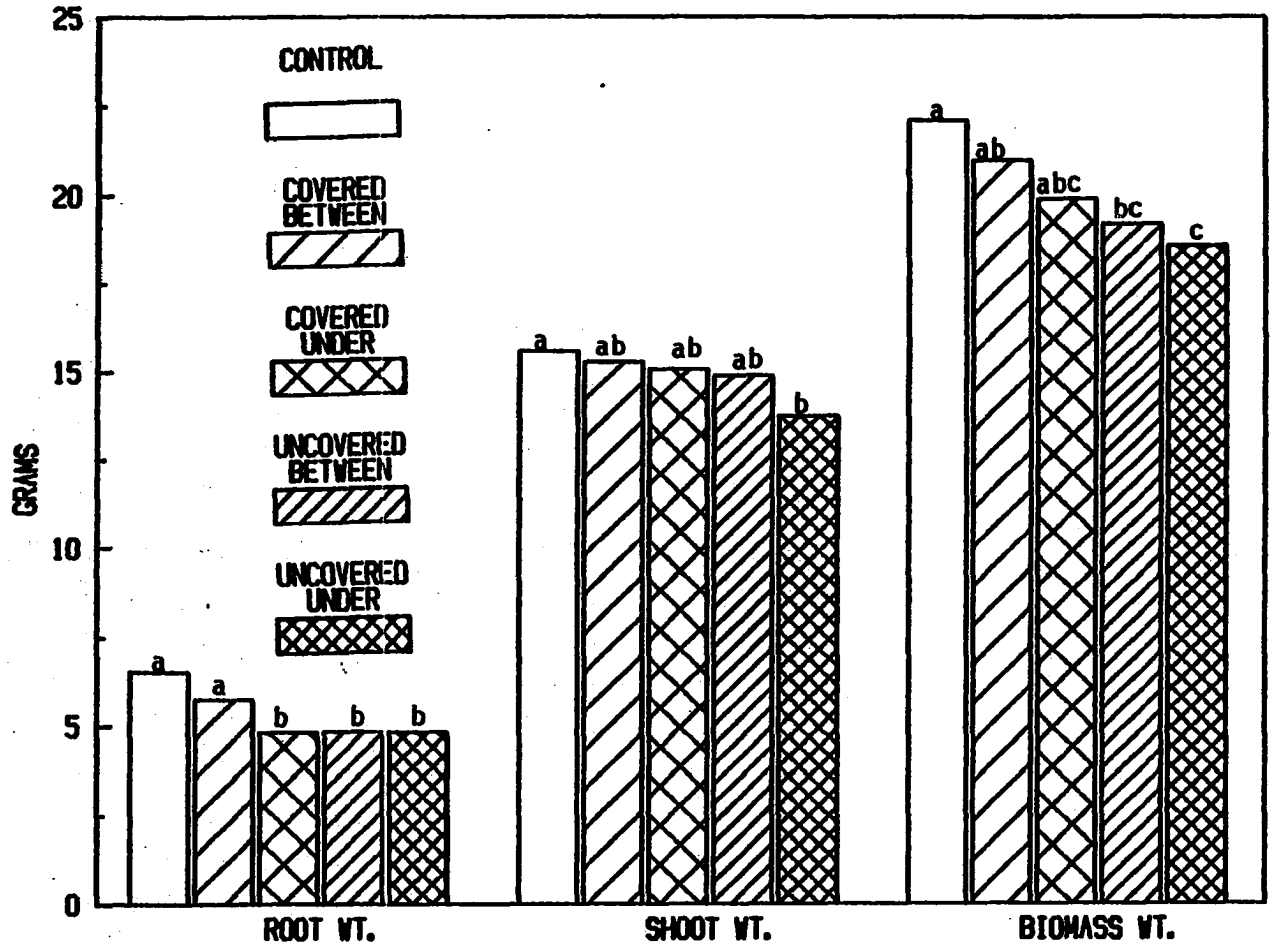

Figure 24. Fresh root, shoot, and biomass weights of corn four weeks after planting grown from soils collected from a corn field; bars with different letters in each category are significantly different

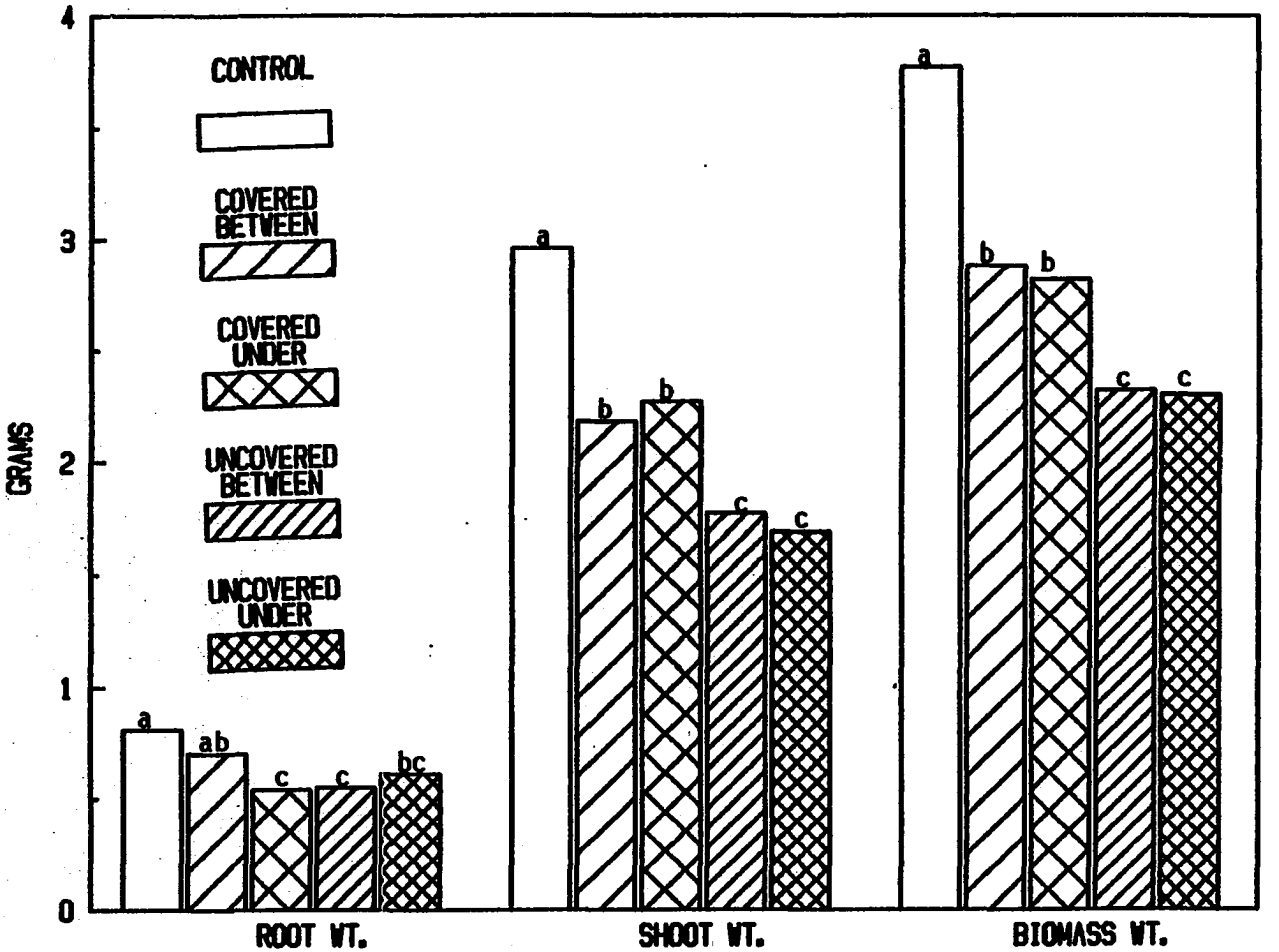

Figure 25. Dry root, shoot, and biomass weights of corn four weeks after planting grown from soils collected from a corn field; bars with different letters in each category are significantly different

The experimental design which was then used was the split-plot design. Tillage practice was the main plot and the extract concentration as the split-plot. Shown in Appendix Tables A12a,b, A13a,b, A14a,b, A15a,b, A16a,b, and A17a,b are the cress seed germination index, germination rate, maximum germination, and onset of 1% germination for the different tillage practices and the different extract concentrations. For all months, the germination index and the onset of 1% germination showed significant differences between the different extract concentrations (Appendix Tables A12b to A17b). This means that the extract concentrations that were used showed allelopathic response.

Significant differences in germination index between the different tillages were observed in April and August (Table 24). During the month of April, the control treatment had a higher germination index of 9440 $(\%\cdot day^{-1})^2$ than the no-till, disc, and plow, which were 8317, 8012, and 7480 $(\%\cdot day^{-1})^2$, respectively. In August, the germination index of the control treatment, 8971 $(\%\cdot day^{-1})^2$, was significantly higher than the no-till, disc, and plow treatments, 8463, 7762, and 8808 $(\%\cdot day^{-1})^2$, respectively.

Comparing the cress seed germination rate of the different tillages during the different months, only the June soil sample gave a significant difference (Table 25). The maximum percentage germination and the onset of 1% germination for the

Table 24. Cress seed germination index $(\%\cdot day^{-1})^2$ using resin column aqueous extracts with different tillage practices from April to September 1982 collected soil samples

Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	9440a	10739a	8152a	7111a	8971a	11387a
No-till	8317ab	9678a	8656a	7004a	8463b	9944a
Disc	8012b	8631a	8737a	7173a	7762c	10653a
Plow	7480b	8457a	8621a	7181a	8088c	10156a

Table 25. Cress seed germination rate $(\%\cdot day^{-1})$ using resin column aqueous estracts with different tillage practices from April to September 1982 collected soil samples

Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	96a	122a	110a	86a	113a	137a
No-till	93a	106a	108ab	97a	104a	107a
Disc	94a	107a	96b	99a	102a	122a
Plow	92a	107a	98ab	99a	100a	120a

different months did not show statistical differences between the different tillages (Tables 26 and 27).

Although not many significant differences were observed in these series of experiments, their results seemed to indicate monthly variations of the parameters measured for the different tillages. In order to observe more clearly the monthly variation in the cress seed germination index, the germination rate, the onset of 1% germination, and the

Table 26. Cress seed maximum percentage germination using resin column aqueous extracts with different tillage practices from April to September 1982 collected soil samples

Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	94a	94a	93a	93a	94a	94a
No-till	91b	92a	96a	93a	94a	94a
Dixc	93a	93a	93a	91a	92a	92a
Plow	91b	93a	93a	91a	94a	94a

Table 27. Onset of one % cress seed germination (in days) using resin column aqueous extracts with different tillage practices from April to September 1982 collected soil samples

Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	0.98a	1.08a	1.15a	1.14a	1.19a	1.01a
No-till	1.08a	1.06a	1.23a	1.32b	1.40a	1.15a
Disc	1.14a	1.21a	1.20a	1.27b	1.14a	1.07a
Plow	1.20a	1.24a	1.09a	1.26b	1.18a	1.15a

maximum cumulative germination, these parameters were all expressed as a percentage of control. The most pronounced allelopathic effect was demonstrated by the cress seed germination index and germination rate. In terms of germination index, expressed as a percentage of control, the no-till, disc, and plow treatments showed inhibitory effects during the months of April, May, August, and September (Table 28 and Figure 26). Stimulatory responses were observed during the

Table 28. Cress seed germination index expressed as % of control using resin column aqueous extracts with different tillage practices from April to September 1982 collected soil samples

Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100a	100	100	100	100a	100a
No-till	88ab	90	106	102	94b	88a
Disc	85b	80	107	102	87c	94a
Plow	79b	79	106	103	90c	90a

months of June and July. The same responses were observed in cress seed germination rate (Table 29 and Figure 27). Inhibitory effects were demonstrated by the different tillages during the months of April, May, August, and September. However, stimulatory responses were obtained for June and July.

To demonstrate the monthly variation in allelopathic effect of corn residue soils, the different tillages were combined together and the cress seed germination index and germination rate were analyzed. As shown in Table 30, germination index for June and July (105 and 102%, respectively) was significantly different from April, May, August, and September (88, 88, 95, and 93%, respectively). Germination rate for June and July (107 and 111%, respectively) was significantly different from May, August, and September (91, 92, and 90%, respectively).

It also was observed that it took a longer time to attain 1% cress seed germination for the no-till, disc, and plow

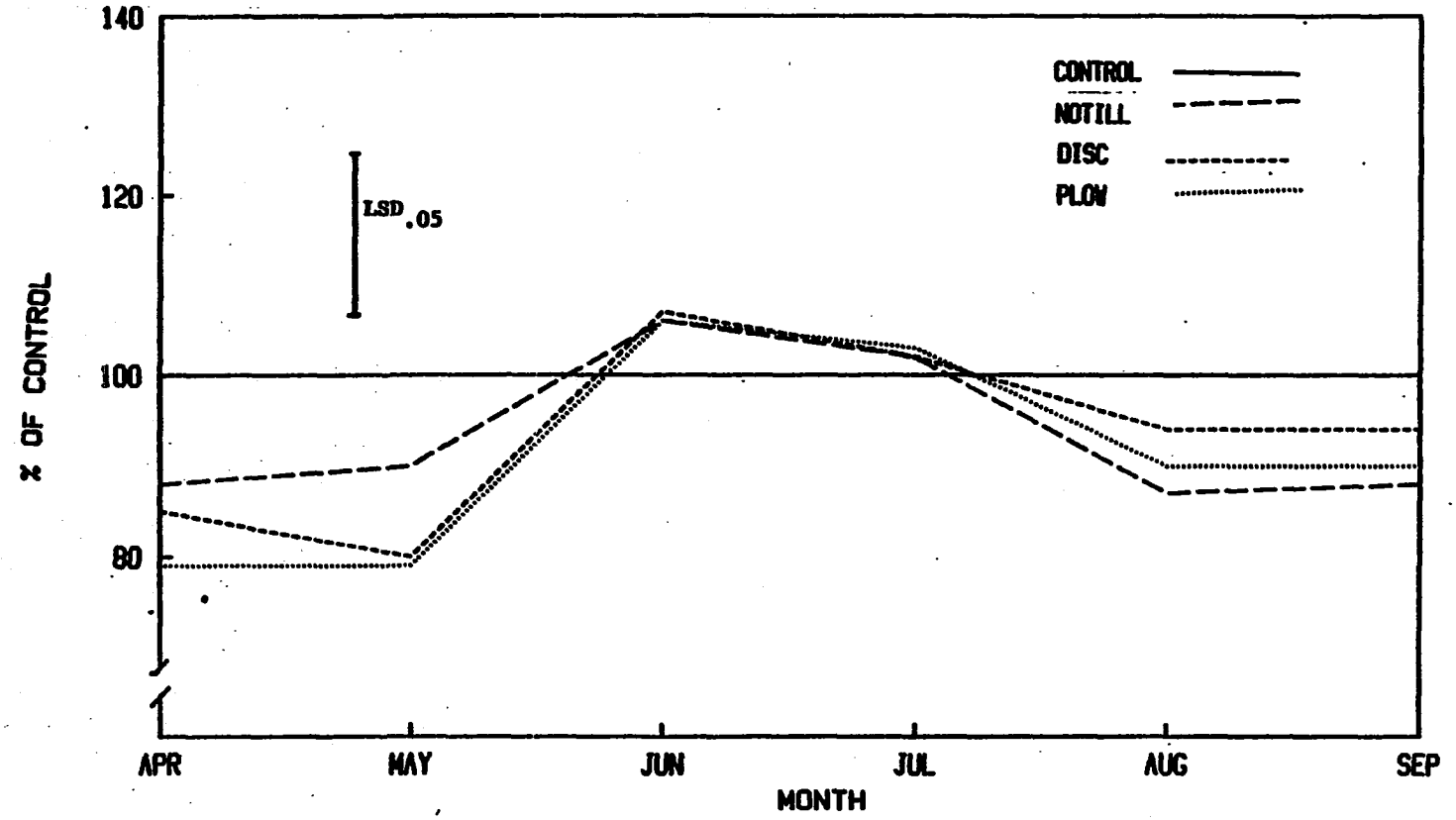

Figure 26. Seasonal variation in cress seed index expressed as % of control using aqueous extract from soil samples with three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

Table 29. Cress seed germination rate expressed as % of control using resin column aqueous extracts with different tillage practices from April to September 1982 collected soil samples

Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100a	100a	100a	100a	100a	100a
No-till	97a	87a	113ab	113a	92a	80a
Disc	98a	88a	115b	115a	90a	90a
Plow	96a	88a	103ab	115a	88a	89a

treatments as compared with the control (Table 31, Appendix Tables A12b to A17b). The maximum cumulative germination was not affected by the different treatments (Appendix Tables A12a to A17a).

The extract concentrations, expressed as percentage of control, required to give a 50% reduction of the index values (I_{50}) for the different tillages are shown in Table 32. Although no statistical significance was observed, the data indicate that the plow treatment required lesser amounts of extract to exhibit 50% reduction (I_{50}) as compared with the control. This was observed for the months of April, May, July, August, and September (76, 53, 78, 95, and 82, respectively). However, for the month of June, it took more extract, 28% more, than the control treatment to have a 50% reduction in index values. The mean for the no-till and disc treatments did not show inhibitory response for all months. The

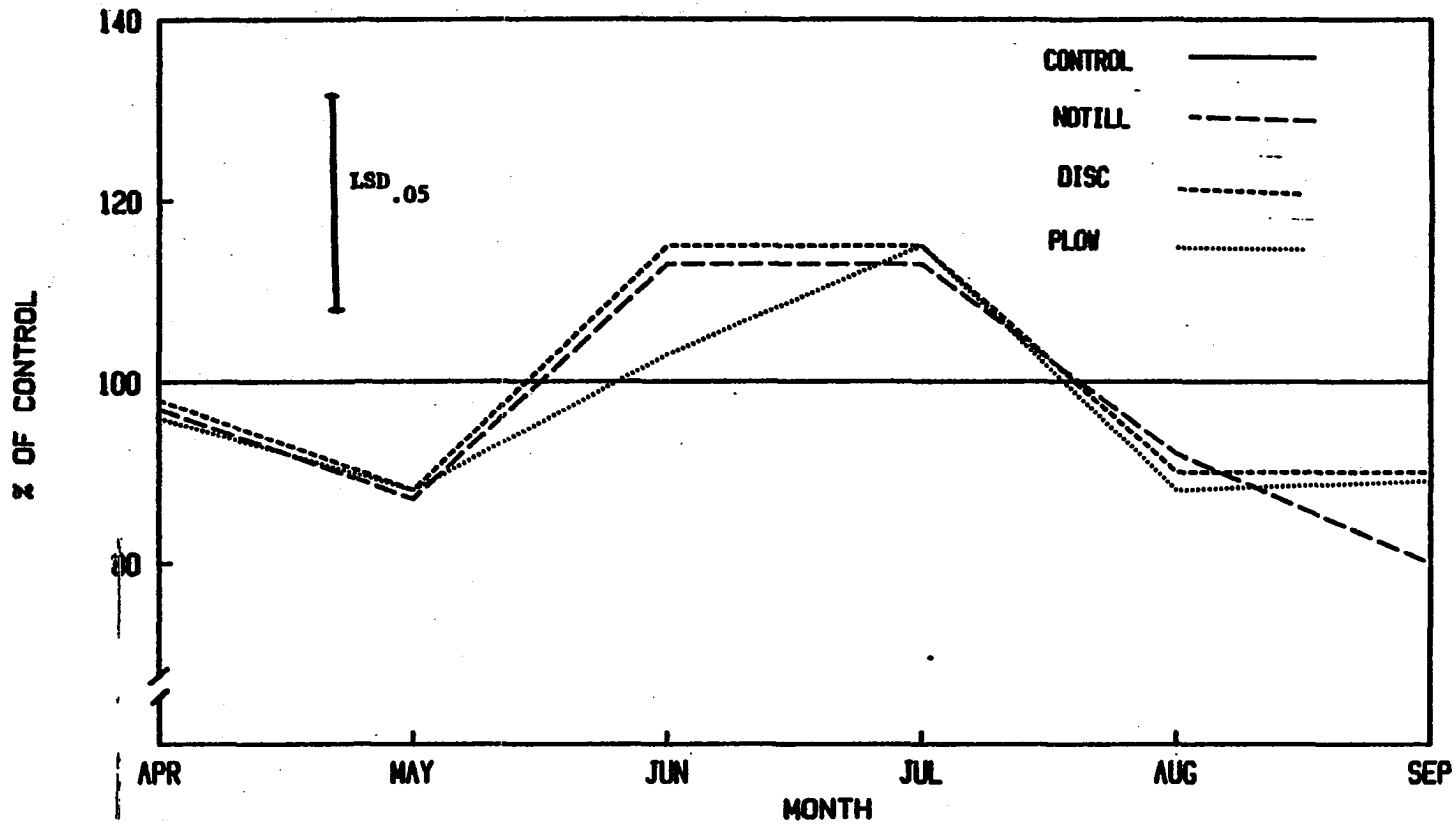

Figure 27. Seasonal variation in cress seed germination rate expressed as % of control using aqueous extract from soil samples with three different tillage practices collected from April to September 1982; monthly soil samples were collected every 25th to 28th day of the month

Table 30. Cress seed germination index and germination rate expressed as % of control using resin column aqueous extracts of corn residue soil collected from April to September 1982

	Apr	May	Jun	Jul	Aug	Sep
Germination index	88c	88c	105a	102ab	95b	93bc
Germination rate	98ab	91b	107a	111a	92b	90b

Table 31. Onset of one % cress seed germination expressed as % of control using resin column aqueous extracts with different tillage practices from April to September 1982 collected soil samples

Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100	100	100	100	100	100
No-till	111	98	107	116	118	113
Disc	117	111	105	111	96	106
Plow	122	114	95	111	100	113

Table 32. Extract concentration expressed as % of control that gave 50% reduction in index values (I_{50}) for the different tillage practices using soil samples collected from April to September 1982

Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	100	100	100	100	100	100
No-till	101	55	116	191	97	78
Disc	107	113	140	364	114	182
Plow	76	53	128	78	95	82

absolute values of the extract concentrations that gave a 50% reduction in index values (I_{50}) are shown in Appendix Table A18.

DISCUSSION

The results of the growth chamber experiments showed that soils which were previously planted to corn and that had corn residues incorporated after harvest had allelopathic effects on growth of corn seedlings. The effects are readily observed by corn seedlings during four weeks of growth. This is clearly shown in Experiments 1 and 2 wherein corn-residue soil had shorter plant height and lower fresh and dry root weights as compared with the control treatment (fallow soil). In Experiment 1, shoot weight, biomass weight, and root:shoot ratio were also affected. However, results of Experiment 1 indicated that the major allelopathic effect was not due to the above-ground plant parts since in the treatments of fallow soil plus fine and coarse corn residues growth was not inhibited. In fact, the fallow soil plus coarse corn residues treatment gave a stimulatory response in terms of plant height, shoot and biomass weights.

Some reports indicate that the inhibitory effects are not due to allelopathy but due to nitrogen immobilization. The result of Experiment 2, however, showed that nitrogen level was not the limiting factor. Even by varying the nitrogen rates from 100 to 400 kg N/ha, no significant differences were observed. The addition of more nitrogen in the corn-residue-soil treatment did not alleviate the decrease in root weight as compared with the control.

Experiments 3 to 8 demonstrated the seasonal variation in allelopathic effects of the three different tillages (no-till, disc, and plow) which were previously and currently planted to corn. The most pronounced effect was on fresh and dry root weights of corn. Note that I will use root weight to emphasize the monthly variation in allelopathic effects on corn growth. This is because root weight was the parameter most affected. However, seasonal variations were also observed in the other parameters (plant height, shoot weight, and biomass weight) that were measured.

For the April sample, the soil from the no-till, disc, and plow treatments inhibited root weight as compared to the control. The no-till treatment was the most inhibited followed by plow then disc treatments. The plants in the no-till treatment were yellow up to the time they were harvested, which was four weeks from planting. A reason why the no-till treatment was most inhibited at this time could be that since most of the corn residues were in the upper 5 cm of the soil surface, the concentration of allelopathic compounds which are being released during decomposition could be higher. The study by Varley and Cruse (1982) showed that corn residues near or with the seed inhibited corn seedling growth.

For the late May sample, the three tillages were no longer inhibitory as in April. Comparing the recovery from the allelopathic effect, the no-till treatment seemed to have

recovered much faster than the plow and disc treatments. The plow treatment was the most inhibitory (Tables 16 and 17, Figures 15 and 16). However, during the months of June and July, a stimulatory response was exhibited by the three tillages. For the month of June, the greatest stimulatory effect was observed in the no-till treatment. The plow treatment was the lowest.

McCalla et al. (1962) reported that microorganisms were more numerous within the top 2.5 cm of soil where residues are conserved. Doran (1981) and Doran et al. (1982) reported that microbial biomass level and population of microorganisms in the surface 50 to 75 mm of no-till soils average 30 to 50% higher than in conventional tillage. Carter and Rennie (1982) also observed that, at 5 to 8 cm depth in no-till, microbial biomass was enriched. Since decomposition of crop residues is hastened by microbial activity, increased microbial biomass would mean faster rate of decomposition under optimum conditions. Although intermediate products during the decomposition process are toxic (Henderson and Farmer, 1955), the presence of numerous microorganisms, which attack and breakdown the allelopathic compounds, would play an important role. Several microorganisms, mostly fungi and bacteria, were found to decompose and break down allelopathic compounds (Tack et al., 1972; Kunc, 1971; Turner and Rice, 1975; Black and Dix, 1976; Henderson and Farmer, 1955; Henderson, 1956). Since

in the no-till treatment a large amount of microbial biomass was present, faster rate of decomposition and breakdown of allelopathic compounds are expected. This could explain why the no-till treatment had the faster rate of recovery from inhibition by May and the greatest stimulatory effect in June and July. The faster decomposition rate of plant residue in the no-till treatment would mean more organic matter to the soil. The slow recovery of the plow treatment from the inhibitory effect shown in April could be due to the reduced number of microorganisms that would break down the allelopathic compounds. Since corn residues in the plow treatment were incorporated up to 20 cm deep in the soil, decomposition rate at this depth could be slow.

During the months of August and September, the inhibitory effects were again observed in all three tillage treatments (Figures 15 and 16). The no-till treatment was less inhibitory than the disc and plow treatments. The inhibitory effects during August and September probably did not come from decomposing corn residues in the field since stimulatory effects had been observed during the months of June and July. Since there were corn plants growing in the field where soil samples were collected and used in the bioassay, the possible source of these allelopathic substances could have come from the existing corn plants. Experiment 9 was then conducted to verify whether growing corn plants release allelopathic sub-

stances and where it is coming from.

The result of Experiment 9 revealed that there was a significant difference in plant height, root weight, shoot weight, and biomass weight between the control treatment (fallow soil) and the covered and uncovered treatments. The soils that were used in the bioassay did not have corn residues because the previous crop had been soybeans. The only possible source of the allelopathic substances from the covered treatments would be exudates from the living roots, whereas for the uncovered treatments, the possible sources would be from rain-leached substances, senescing plant parts and root exudates. According to Rovira (1969) and Rovira et al. (1979), root exudates are low molecular weight compounds which are released into the surrounding medium by living and intact roots. Under normal growth conditions, root exudation probably represents a major mechanism of releasing organic chemicals into the rhizosphere. As shown in Table 23 and Figure 25, the biomass weight for the control was significantly different from the covered and uncovered treatments. The difference in biomass weight between the control and the covered treatments would then be due to allelopathic substances exuded by the roots. The difference in biomass weight between the covered and uncovered treatments would then be due to rain-leached substances and senescing plant parts. This study clearly indicates that allelopathic substances are released

by plants during their growth. We could also say then that the inhibitory effect observed in August and September was due to the allelopathic substances released by the current corn crop.

A study by Jimenez et al. (1982) revealed that corn pollen exhibited strong radicle inhibition in a corn bioassay. The pollen could be a strong source of allelopathic compounds released during anthesis. As shown in Figure 16, after the stimulatory effect in June and July, an inhibitory response was again reflected in August and September. This inhibitory response coincided after the pollen had been shed, which could suggest that the pollen may be a source of this allelopathic compound. Note that in Experiment 9, the uncovered treatments were more inhibitory than the covered, which could implicate pollen as a source of allelopathic substances. It could also be argued that the inhibitory effect of the different tillages during the month of April could be a carry-over of the allelopathic substances deposited by rain-leached substances, senescing plant parts, and root exudates from corn plants of the previous crop and not due purely to corn residues left after harvest.

As mentioned earlier, the most pronounced allelopathic effects were seen on root weights. When the root:shoot ratio was compared between the control (fallow soil) and corn residue soil treatment, the latter showed significantly lower

root:shoot ratio (Tables 2, 3, 5, and 6). This means that the effect of the allelopathic substances would be more on the roots rather than in the shoot. In Experiments 3 to 8, however, the root:shoot ratio of the control and the plow treatment were not different from each other in all months, except for August (Appendix Tables A2c to A7c). On the other hand, root:shoot ratio of the disc and no-till treatments were lower than the control and the plow. This suggests that, although the root and shoot weight of the plow treatment is lower than the control, the rate of root growth is proportional to the rate of shoot growth for both treatments. In other words, both root and shoot were severely affected in the plow treatment. However, for the disc and no-till treatments, which had lower root:shoot ratio, the roots were inhibited more than the shoot.

The results of the resin column experiments agree with the results obtained in Experiments 3 to 8. Using cress seed germination index (I) and germination rate (R) to show the monthly variation in allelopathic effects for the different tillages, inhibitory effects were observed for April, May, August, and September, while stimulatory effects were seen for June and July (Figures 26 and 27). On the other hand, maximum germination (A) did not show any allelopathic response for the different tillages. It also was observed that, in the no-till, disc, and plow treatments, it took a longer time

to attain 1% germination ($t_{0.01A}$) as compared with the control (Table 31). This would indicate that allelopathic substances are present in the extracts.

The technique of indexing the three cumulative seed germination parameters, as used by Lehle and Putnam (1982), permits the use of seed germination as a bioassay to quantify the toxicity of plant extracts. The use of a numerical index derived from the three germination parameters (A, R, and $t_{0.01A}$) quantified the allelopathic effects better than any of the individual parameters of seed germination.

The results of the cress seed germination bioassay indicated that inhibitory substances were effectively trapped by the XAD-4 resin column. Amberlite XAD-4 is a hydrophobic styrene-divinyl benzene co-polymer with a specific surface area of 750 m^2/g. With the physical and chemical characteristics of XAD-4, it makes it ideal to extract allelopathic compounds from the recirculating solution. Most known allelopathic compounds are secondary plant metabolites including phenolic acid and flavonoids and other aromatics, terpenoids, steroids, alkaloids, and organic cyanides (Whittaker and Feeny, 1971; Chou and Patrick, 1976; Pareek and Gaur, 1973; Guenzi and McCalla, 1966; Rasmussen and Einhellig, 1980). Since most of these compounds are sufficiently hydrophobic, they could easily be trapped by XAD-4. The results also showed that most of the allelopathic compounds in the

recirculating solution could be easily trapped by XAD-4 resin column even by just attaching the column to the circulating mechanism for one day.

The results of all the experiments conducted explicitly reveal that living corn plants (root exudates and rain-leached substances) and corn plant residues left in the fields after harvest release allelopathic substances which inhibit subsequent corn growth, especially the root, and cress seed germination.

SUMMARY AND CONCLUSIONS

The growth chamber experiments revealed that soils which were previously planted to corn and had corn residues left after harvest had strong allelopathic effect on corn seedling growth. As observed in Experiment 1, corn grown in soils previously planted to corn and with corn residues left after harvest had shorter plant height, lower fresh and dry root, shoot, and biomass weights as compared with the control (fallow soil). However, in Experiment 2, only the plant height and the fresh and dry root weights were significantly affected by the soil with corn residues.

Results of Experiment 2 showed that the inhibitory effect was due to allelopathy and not because of nitrogen immobilization. Even by varying the nitrogen rates from 100 to 400 kg N/ha, no significant differences were observed. The addition of more nitrogen in the corn-residue soil treatment did not alleviate the decrease in root weight as compared with the control.

In Experiments 3 to 8, soil samples from a previously planted corn field and with corn currently growing were collected every month from April to September, 1982, for corn bioassay. The soil samples which were collected constituted different tillage practices (no-till, disc, and plow). The plants from the soils with corn residues had shorter plant height, smaller root, shoot, and biomass weights than

the control (fallow soil). The most pronounced effect was on root weight. The April, August, and September soil samples were inhibitory to corn growth, whereas June and July soil samples had stimulatory effects. The April soil sample for the no-till treatment was the most inhibitory and the plants were yellowish in color. However, the late May soil samples for the three tillage practices were no longer inhibitory as compared to the April soil samples. The no-till treatment seemed to have recovered much faster than the disc and plow treatments. The June and July soil samples for the three tillages exhibited a stimulatory response. The greatest stimulatory effect was observed in the no-till treatment, while the lowest was observed in the plow treatment. Since the inhibitory effect of August and September soil samples probably did not come from chemicals released from the decomposing corn residues, Experiment 9 was conducted to verify whether growing corn plants releases allelopathic substances and where it was coming from.

The results of Experiment 9 revealed that allelopathic substances were being released by living corn plants through root exudation, rain-leached substances, and senescing aboveground plant parts.

Using XAD-4 resin to trap allelopathic substances, the monthly soil samples collected in Experiments 3 to 8 were attached to a circulating mechanism with XAD-4 resin column.

The extracts from the column were bioassayed using cress seed germination. Cress seed germination index, germination rate, onset of 1% germination, and maximum germination percentage were derived using the SAS nonlinear regression program and the Richards' function. The results showed that germination index and germination rate were inhibited by the April, May, August, and September soil samples but were stimulated by the June and July soil samples.

The results of all the experiments conducted lead to the conclusion that living corn plants (root exudates and rain-leached substances) and corn plant residues left after harvest release allelopathic substances which inhibit subsequent corn growth, especially the root, and cress seed germination.

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ACKNOWLEDGMENTS

I wish to express my deepest gratitude to my major professor, Dr. Irvin C. Anderson, for his invaluable guidance, advice and understanding, for his continuous support and friendship throughout the course of my graduate studies at Iowa State University, and for his constructive criticism during the writing of this manuscript. His friendship, guidance and support will always be remembered and appreciated.

I also would like to express my gratitude to the other members of my graduate committee, Drs. Richard M. Cruse, Clifford E. LaMotte, Robert H. Shaw and David K. Whigham for their guidance.

I am very deeply indebted to Dr. Arturo Gomez and Dr. Edgardo Quisumbing for helping me find scholarship support.

I am thankful to USAID for funding my graduate studies and to the International Development Research Center for supporting my travel to the United States. Sincerest appreciation is also extended to Dr. William J. Jorns of the International Agricultural Programs at Kansas State University and to Ms. Verlee Clinefelter of the International Agricultural programs at Iowa State University for their help in managing my scholarship and for extending their valuable help during my time of need.

I am grateful to Mrs. Ina Couture for her assistance in typing my manuscript. A special note of appreciation is extended to all my friends in the Filipino Student Association and to all the graduate students in my group for their help, encouragement and for making my stay here at ISU a pleasant one.

Most of all, I am very grateful to my parents, Romeo and Emeteria, my brother, sisters, nephew and nieces for their understanding, encouragement and prayers throughout the period of my graduate studies. To my aunt, Elizabeth, Advincula, and her family and to my uncle, Emerito Garcia, and his family for their help and concern for me during my stay here in the United States.

APPENDIX

Table A1. Initial values and allowable ranges of Richards' function parameters specified in SAS nonlinear regression program (after Lehle and Putnam, 1982)

Parameter	Initial value	Allowable range
	m > 1 and m = 1.001	
A	85-95	0-100
b	0.5-3.5	0-1000
k	2.4	0-100
m	1.001	1 < m < 10
	m < 1	
A	70-95	0-100
b	0.002-0.02	0-1000
k	2.322-3.473	0-100
m	0.9999	0 < m < 1

Table A2a. Plant height of corn seedlings one, two, three, and four weeks after planting using April 1982 collected soil samples with different tillage practices

Tillage	Plant height (cm)			
	Week 1	Week 2	Week 3	Week 4
Control	14a	43a	70a	94ab
No-till	15a	42a	68a	95ab
Disc	15a	43a	71a	99a
Plow	13a	40a	67a	90b

Table A2b. Fresh root weight, shoot weight, biomass weight and root:shoot ratio four weeks after planting using April 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	(g)			
Control	6.05a	18.13b	24.18a	0.33a
No-till	4.05c	17.76b	20.81b	0.24b
Disc	5.16ab	21.56a	26.72a	0.24b
Plow	4.81bc	13.44c	18.25b	0.36a

Table A2c. Dry root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using April 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	(g)			
Control	0.49a	1.59b	2.08ab	0.30a
No-till	0.34b	1.42bc	1.76bc	0.24b
Disc	0.43ab	1.97a	2.41a	0.22b
Plow	0.39ab	1.23c	1.62c	0.31a

Table A3a. Plant height of corn seedlings one, two, three and four weeks after planting using May 1982 collected soil samples with different tillage practices

	Plant height (cm)			
Tillage	Week 1	Week 2	Week 3	Week 4
Control	7a	39a	68ab	96b
No-till	6ab	39a	72a	101b
Disc	7a	38a	69a	101a
Plow	4b	36a	64b	92c

Table A3b. Fresh root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using May 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
Control	8.21ab	21.36b	29.59b	0.39a
No-till	8.79a	26.22a	35.01a	0.34b
Disc	9.16a	27.01a	36.17a	0.34b
Plow	7.41b	18.11a	25.52c	0.41a

Table A3c. Dry root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using May 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
Control	1.18a	2.57b	3.75b	0.46a
No-till	1.12a	2.84ab	3.96ab	0.40b
Disc	1.20a	3.39a	4.58a	0.37b
Plow	1.11a	2.39b	3.51b	0.47a

Table A4a. Plant height of corn seedlings one, two, three, and four weeks after planting using June 1982 collected soil samples with different tillage practices

	Plant height (cm)			
Tillage	Week 1	Week 2	Week 3	Week 4
Control	7b	43c	72c	95c
No-till	9a	47a	88a	110c
Disc	9a	43bc	78b	104b
Plow	8ab	46ab	79b	102b

Table A4b. Fresh root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using June 1982 collected soil samples with different tillage practices

Tillage	Root weight (g)	Shoot weight (g)	Biomass weight (g)	Root: shoot ratio
Control	6.86bc	21.96c	28.80c	0.31a
No-till	8.75a	37.40a	46.16a	0.23c
Disc	7.93ab	28.40b	36.33b	0.28ab
Plow	6.58c	25.73b	32.30c	0.26bc

Table A4c. Dry root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using June 1982 collected soil samples with different tillage practices

Tillage	Root weight (g)	Shoot weight (g)	Biomass weight (g)	Root: shoot ratio
Control	0.67b	2.14c	2.81c	0.31a
No-till	0.83a	3.49a	4.32a	0.24b
Disc	0.75b	2.67b	3.42b	0.28a
Plow	0.69b	2.45bc	3.14bc	0.28a

Table A5a. Plant height of corn seedlings one, two, three, and four weeks after planting using July 1982 collected soil samples with different tillage practices

	Plant height (cm)			
Tillage	Week 1	Week 2	Week 3	Week 4
Control	6ab	39ab	74b	92b
No-till	8a	43a	84a	99a
Disc	3b	36b	70c	92b
Plow	8a	40ab	71bc	92b

Table A5b. Fresh root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using July 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
Control	7.11b	17.17bc	24.28b	0.42ab
No-till	8.71a	26.77a	35.48a	0.33c
Disc	7.71ab	18.98b	26.69b	0.41b
Plow	7.77ab	16.96c	24.73b	0.46a

Table A5c. Dry root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using July 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
Control	0.75ab	1.94b	2.69b	0.39a
No-till	0.85a	2.87a	3.72a	0.30c
Disc	0.73b	2.08b	2.81b	0.35b
Plow	0.79ab	1.95b	2.73b	0.40a

Table A6a. Plant height of corn seedlings one, two, three, and four weeks after planting using August 1982 collected soil samples with different tillage practices

	Plant height (cm)			
Tillage	Week 1	Week 2	Week 3	Week 4
Control	13a	44a	73a	94ab
No-till	9b	36c	68b	98a
Disc	9b	36c	65b	93bc
Plow	12a	40b	68b	91c

Table A6b. Fresh root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using August 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	(g)			
Control	7.49b	22.41b	29.90b	0.33a
No-till	8.49a	26.44a	34.93a	0.32a
Disc	6.39c	19.16c	25.56c	0.34a
Plow	5.69c	18.82c	22.50c	0.34a

Table A6c. Dry root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using August 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	(g)			
Control	0.76a	3.86a	4.63a	0.21b
No-till	0.75a	4.20c	4.95a	0.20b
Disc	0.57b	1.98b	2.55b	0.29a
Plow	0.57b	1.93b	2.50b	0.30a

Table A7a. Plant height of corn seedlings one, two, three, and four weeks after planting using September 1982 collected soil samples with different tillage practices

	Plant height (cm)			
Tillage	Week 1	Week 2	Week 3	Week 4
Control	5a	34a	63a	86ab
No-till	5a	34a	65a	87a
Disc	5a	34a	64a	80bc
Plow	5a	34a	60a	76c

Table A7b. Fresh root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using September 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
Control	5.97a	13.96b	19.93ab	0.43a
No-till	5.06b	16.13a	21.19a	0.31c
Disc	5.03b	12.88bc	17.90bc	0.39ab
Plow	4.78b	11.40c	15.68c	0.37b

Table A7c. Dry root weight, shoot weight, biomass weight, and root:shoot ratio four weeks after planting using September 1982 collected soil samples with different tillage practices

Tillage	Root weight	Shoot weight	Biomass weight	Root: shoot ratio
	----------(g)----------			
Control	0.56a	1.85b	2.41a	0.31a
No-till	0.48ab	2.29a	2.77a	0.21b
Disc	0.49ab	1.39c	1.88b	0.36a
Plow	0.43b	1.20c	1.63b	0.36a

Table A8. F values obtained from cress seed germination index analysis of variance

Source of variation	d.f.	Month					
		Apr	May	Jun	Jul	Aug	Sep
Rep	1	0.13	126.37**	6.20	3.90	0.71	7.60
Tillage (T)	3	2.4	8.22*	2.10	0.43	3.93	0.12
Error a	3						
Time	2	11.11*	7.86*	78.95**	9.64*	27.82**	6.09*
Time*T	6	3.25	1.93	4.88*	0.68	1.79	1.31
Error b	8						
Concentration (C)	3	59.38**	13.16**	96.35**	2.22	29.22**	32.31**
T*C	9	1.70	0.76	0.96	0.83	0.96	1.08
Time*C	6	4.95**	3.16**	7.48**	2.24	1.85	1.94
T*time*C	18	1.12	0.68	1.67	0.74	0.71	1.76
Error c	36						

*,**Significant at the 0.05 and 0.01 levels of probability, respectively.

Table A9. F values obtained from cress seed onset of 1% germination analysis of variance

Source of variation	d.f.	Month					
		Apr	May	Jun	Jul	Aug	
Rep	1	0.00	3.54	9.50	0.03	0.30	0.64
Tillage (T)	3	3.88	1.49	0.29	3.01	0.99	1.61
Error a	3						
Time	2	32.26**	14.47**	61.69**	26.36**	9.15**	7.94**
Time*T	6	2.35	0.91	2.71	2.18	0.41	1.01
Error b	8						
Concentration (C)	3	42.67**	13.74**	9.76**	23.71**	5.43*	17.71**
T*C	9	1.57	1.21	1.10	1.03	0.95	0.99
Time*C	6	8.67**	5.33**	13.24**	2.73*	1.84	5.22**
T*time*C	18	1.13	0.86	0.88	0.96	1.08	1.59
Error c	36						

*,**Significant at the 0.05 and 0.01 levels of probability, respectively.

Table A10. F values obtained from cress seed germination rate analysis of variance

Source of variation	d.f.	Month					
		Apr	May	Jun	Jul	Aug	Sep
Rep	1	0.07	8.62*	0.80	2.82	0.09	6.68
Tillage (T)	3	0.06	0.38	0.84	1.15	0.31	0.05
Error a	3						
Time	2	0.81	0.56	14.38**	0.66	0.07	0.66
Time*T	6	2.01	1.57	4.20*	0.99	1.36	1.41
Error b	8						
Concentration (C)	3	5.55**	2.59*	33.59**	3.45*	3.63*	13.21**
T*C	9	0.79	1.05	1.04	0.98	1.01	0.61
Time*C	6	2.82*	0.96	0.59	0.96	0.88	0.39
Time*T*C	18	1.04	0.56	1.02	0.94	1.09	1.49
Error c	36						

*,**Significant at the 0.05 and 0.01 levels of probability, respectively.

Table A11. F values obtained from cress seed maximum germination analysis of variance

Source of variation	d.f.	Month					
		Apr	May	Jun	Jul	Aug	Sep
Rep	1	0.75	39.04**	3.18	1.41	0.02	54.95**
Tillage (T)	3	18.44**	7.08*	0.98	2.30	0.44	0.28
Error a	3	1.32	2.62	0.13	2.45	0.05	0.01
Time*T	6	2.68	0.79	1.46	0.87	3.27	1.06
Error b	8						
Concentration (C)	3	9.08**	0.36	14.30**	9.63**	5.99*	0.66
T*C	9	1.96	0.27	0.51	0.49	0.83	1.00
Time*C	6	2.64	0.78	0.17	1.35	1.02	0.79
T*time*C	18	1.45	1.32	0.61	1.03	0.91	1.29
Error c	36						

*,**Significant at the 0.05 and 0.01 levels of probability, respectively.

Table A12a. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using resin column aqueous extracts with different tillage practices from April 1983 collected soil samples

Tillage	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
Control	9440a	96a	94a	0.98a
No-till	8317ab	93a	91b	1.08a
Disc	8012b	94a	93a	1.14a
Plow	7480b	92a	91b	1.20a

Table A12b. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using varying concentrations of resin column extract from April 1982 collected soil samples

Extract (ml)	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
0	10979a	105a	94a	0.90a
0.2	8577b	94a	92ab	1.00b
0.4	8583b	100a	94a	1.10bc
2.0	5010b	76b	90b	1.40c

Table A13a. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using resin column aqueous extracts with different tillage practices from May 1982 collected soil samples

Tillage	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
Control	10739	122	94	1.08
No-till	9678	106	92	1.06
Disc	8631	107	93	1.21
Plow	8457	107	93	1.24

Table A13b. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using varying concentrations of resin column extract from May 1982 collected soil samples

Extract (ml)	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
0	11865a	120a	93a	0.94b
0.5	10638a	117a	94a	1.05b
1.0	8422b	113a	94a	1.27a
2.0	6578b	92a	93a	1.35a

Table A14a. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using resin column aqueous extracts with different tillage practices from June 1982 collected soil samples

Tillage	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
Control	8152a	110a	93a	1.15a
No-till	8656a	108ab	96a	1.23a
Disc	8737a	96b	93a	1.20a
Plow	8621a	98ab	93a	1.09a

Table A14b. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using varying concentrations of resin column extract from June 1982 collected soil samples

Extract (ml)	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
0	13002a	139a	97a	1.03c
0.5	8105b	93b	93b	1.03c
1.0	7349b	91b	93b	1.18a
2.0	5710c	90b	92b	1.43b

Table A15a. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using resin column aqueous extract with different tillage practices from July 1982 collected soil samples

Tillage	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
Control	7111a	86a	93a	1.14a
No-till	7004a	97a	93a	1.32b
Disc	7173a	99a	91a	1.27b
Plow	7181a	99a	91a	1.26b

Table A15b. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using varying concentrations of resin column extract from July 1982 collected soil samples

Extract (ml)	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
0	7855a	86b	95a	1.03a
0.5	7811a	102a	92b	1.20b
1.0	6844ab	99a	91b	1.32c
2.0	5960b	95ab	90b	1.44d

Table A16a. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using resin column aqueous extract with different tillage practices from August 1982 collected soil samples

Tillage	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
Control	8971a	113a	94a	1.19a
No-till	8463b	104a	94a	1.40a
Disc	7762c	102a	92a	1.14a
Plow	8088c	100a	94a	1.18a

Table A16b. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using varying concentrations of resin column extract from August 1982 collected soil samples

Extract (ml)	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
0	11430a	114a	95a	0.94a
0.5	7238b	93a	92b	1.26ab
1.0	7429b	102a	93ab	1.30ab
2.0	7188b	110a	93ab	1.42b

Table A17a. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using resin column aqueous extract with different tillage practices from September 1982 collected soil samples

Tillage	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
Control	11387	137	94	1.01
No-till	9944	107	94	1.15
Disc	10653	122	92	1.07
Plow	10156	120	94	1.15

Table A17b. Cress seed germination index, germination rate, maximum germination, and onset of 1% germination using varying concentrations of resin column extract from September 1982 collected soil samples

Extract (ml)	Index $(\%\cdot day^{-1})^2$	Germination rate (%·day)	Maximum germination (%)	Onset of 1% germination (days)
0	13927a	147a	94a	0.98a
0.5	11008b	120b	95a	1.02a
1.0	9238c	110b	92a	1.09a
2.0	7966c	109b	93a	1.28b

Table A18. Extract concentration (ml) which gave 50% reduction in index value (I_{50}) for the different tillage practices using soil samples collected from April to September 1982

	Month					
Tillage	Apr	May	Jun	Jul	Aug	Sep
Control	2.15	3.24	1.54	4.64	2.42	2.51
No-till	2.18	1.78	1.79	8.86	2.36	1.95
Disc	2.79	3.67	2.16	16.91	2.77	4.56
Plow	1.64	1.73	1.97	3.64	2.31	2.05

Table A19. SAS nonlinear regression program for m > 1

```
//STEP1 EXEC SAS
//SYSIN DD *
DATE:
INPUT X Y @@;
CARDS:

COMMENT M GREATER THAN ONE;
TITLE CRESS SEED GERMINATION;
PROC NLIN METHOD=MARQUARDT ITER=125;
PARAMETERS A=90  B=1.0  K=2.4  M=1.001;
BOUNDS   1 <=M<10   0<=A<100   0<=K<100   0<=B<1000;
E=EXP(-K*X);  D=1.000/(1.000-M);
F=1+B*E;  G=F**D;
MODEL  Y=A*B;
DER.A=G;
DER.B=A*E*D*(F**(D-1));
DER.K=-A*B*X*D*E*(F**(D-1));
DER.M=A*(LOG(F))*G*(D**2);
```

Table A20. SAS nonlinear regression program for m < 1

```
//STEP1 EXEC SAS
//SYSIN DD *
DATA;
INPUT X Y @@;
CARDS;

COMMENT  M LESS THAN ONE;
TITLE  CRESS SEED GERMINATION;
PROC NLIN METHOD=MARQUARDT  ITER=125;
PARAMETERS  A=90  B=0.1  K=2.4  M=.9999;
BOUNDS  0 <+M<1  0<=A<100  0<=K<100  0<=B<1000;
E=EXP(-K*X);  D=1.000/(1.000-M);
F=1-B*E;  G=F**D;
MODEL  Y=A*G;
DER.A=G;
DER.B=-A*E*D*(F**(D-1));
DER.K=A*B*X*D*E*(F**(D-1));
DER.M=A*(LOG(F))*G*(D**2);
```